UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE ODONTOLOGÍA

TESIS DOCTORAL

Predisposición genética a la reabsorción radicular: GWAS

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

Paula Iber Díaz

DIRIGIDA POR

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A Dios, por hacer todo esto posible

y ponerme a personas tan maravillosas en el camino

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"Si he podido ver lejos, es porque estaba a hombros de gigantes" Isaac Newton.

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PREFACIO

Esta tesis doctoral fue respaldada mediante un contrato laboral docente e investigador de ayudante (0103/AYU/003) *(Anexo III)* en el Departamento de Especialidades Clínicas Odontológicas de la Facultad de Odontología de la Universidad Complutense de Madrid. La presente tesis doctoral se basa en los siguientes cuatro artículos científicos:

Paper #1. Iber-Diaz P, Senen-Carramolino R, Otero-Pregigueiro A, Palma-Fernandez JC, Iglesias-Linares A. (2024). External apical root resorption assessment revisited: a scoping review. [Under review]. *The Angle Orthodontist.*

Paper #2. Iber-Diaz P, Dehesa-Santos A, Senen-Carramolino R, Palma-Fernandez JC, Iglesias-Linares A. (2024). Ethnic differences in root resorption induced by orthodontic forces. [Under review]. *Clinical Oral Investigations.*

Paper #3. Iber-Diaz P, Senen-Carramolino R, Iglesias-Linares A, Fernandez-Navarro P, Flores-Mir C, Yanez-Vico RM. (2020). GWAS of Post-Orthodontic Aggressive External Apical Root Resorption Identified Multiple Putative Loci at X-Y Chromosomes. *Journal of Personalized Medicine*; 10,4. doi: 10.3390/jpm10040169

Paper #4. Iber-Diaz P, Senen-Carramolino R, Fernandez-Navarro P, Palma-Fernandez JC, Iglesias-Linares A (2024). GWAS of post-orthodontic aggressive external apical root resorption. [In preparation]. *Nature Genetics*.

ÍNDICE

1. RESUMEN

La reabsorción radicular apical externa (EARR) es uno de los efectos secundarios iatrogénicos más comunes asociados al tratamiento de ortodoncia, representando un desafío significativo para los ortodoncistas debido a su naturaleza multifactorial y su complejidad en términos de diagnóstico y etiología. Esta tesis doctoral aborda el estudio integral de la EARR desde diversas perspectivas, mediante una serie de publicaciones científicas orientadas a esclarecer las bases diagnósticas, etiológicas y genéticas de esta condición.

El primer capítulo analiza de manera crítica la literatura disponible sobre los métodos radiográficos utilizados para identificar la EARR durante los tratamientos ortodóncicos con aparatología fija, explorando la diversidad en las técnicas de evaluación y los grados de severidad de la condición. Los hallazgos revelan una falta de consenso en las estrategias diagnósticas y enfatizan la necesidad de estandarizar los protocolos para aumentar la precisión en la práctica clínica.

El segundo capítulo examina en profundidad las variaciones étnicas en la susceptibilidad a la reabsorción radicular apical externa agresiva EARR, considerando factores como la morfología apical, la proporción corona/raíz y la duración del tratamiento ortodóncico. Mediante el análisis de una muestra de 200 pacientes de diferentes grupos étnicos, se observó una menor incidencia de EARR leve/moderada y una mayor prevalencia de morfología apical puntiaguda en pacientes latinoamericanos en comparación con los europeos. Sin embargo, no se encontraron diferencias relevantes entre grupos étnicos en las formas más agresivas de EARR. Este estudio subraya la necesidad de incorporar las características étnicas y morfológicas en la planificación de tratamientos ortodóncicos más personalizados.

El tercer capítulo explora las bases genéticas implicadas en el desarrollo de la reabsorción radicular apical externa agresiva (aEARR) a través de dos estudios de asociación del genoma completo (GWAS), complementados con el análisis de factores clínicos y radiográficos. En ambos estudios, se identificaron múltiples variantes genéticas asociadas a esta patología. En el primer estudio, realizado con una muestra de 480 pacientes con estratificación en función de origen étnico, se identificaron variantes en los genes *STAG2*

(rs151184635) y *RP1-30E17.2* (rs55839915), sugiriendo un posible componente genético relacionado con el género. Por otro lado, en el segundo estudio, que incluyó 612 pacientes con inclusión de un modelo multinivel con estratos étnicos múltiples, se destacó la variante genética rs1122449 en el gen *IRX2* como la más significativa en relación con aEARR. Además, se determinaron factores de riesgo significativos asociados con una mayor predisposición a la aEARR, entre ellos, ser hombre, el reposicionado y adhesión repetida de brackets sobre los dientes y la onicofagia. Por otro lado, el uso de brackets con un slot de 0.022" se identificó como un elemento protector frente a esta condición. Estos resultados confirman un perfil genético de riesgo que tiene un carácter étnico-dependiente. Por tanto, podemos concluir que la predisposición a la aEARR es un proceso patológico ligado a una interacción esencial entre factores genéticos y clínicos, con especificidad étnica. Este conocimiento allana el camino para el desarrollo de estrategias terapéuticas personalizadas y de precisión en el ámbito preventivo y terapéutico que se adapten al perfil genético de cada paciente, orientadas a optimizar los tratamientos y reducir el riesgo de patologías secundarias a la intervención, tal y como ocurre en la reabsorción radicular apical externa en ortodoncia.

El análisis global de estos hallazgos proporciona una perspectiva integral de los factores diagnósticos, étnicos y genéticos que influyen en la EARR. En este contexto, la tesis plantea un enfoque hacia tratamientos personalizados, promoviendo el avance hacia una ortodoncia de precisión y un manejo clínico que no solo optimice los resultados, sino que también minimice las complicaciones y mejore la calidad de vida de los pacientes.

2. ABSTRACT

External apical root resorption (EARR) is one of the most common iatrogenic side effects associated with orthodontic treatment, posing a significant challenge for orthodontists due to its multifactorial nature and the complexity of its diagnosis and etiology. This doctoral thesis comprehensively addresses the study of EARR from various perspectives, presenting a series of scientific publications aimed at elucidating the diagnostic, etiological, and genetic foundations of this condition.

The first chapter critically analyzes the available literature on the radiographic methods used to detect EARR during orthodontic treatment with fixed appliances, exploring the variability in evaluation techniques and the severity levels of the condition. The findings reveal a lack of consensus regarding diagnostic strategies and emphasize the urgent need to standardize protocols to enhance precision in clinical practice.

The second chapter thoroughly examines ethnic variations in susceptibility to EARR, considering factors such as apical morphology, crown-to-root ratio, and the duration of orthodontic treatment. Through the analysis of a sample comprising 200 patients from different ethnic groups, a lower incidence of mild to moderate EARR and a higher prevalence of pointed apical morphology were observed in Latin American patients compared to European patients. However, no significant differences between ethnic groups were identified in the more aggressive forms of EARR. This study highlights the importance of integrating ethnic and morphological characteristics into the planning of more personalized orthodontic treatments.

The third chapter explores the genetic bases involved in the development of aggressive external apical root resorption (aEARR) through two genome-wide association studies (GWAS) complemented by the analysis of clinical and radiographic factors. Both studies identified multiple genetic variants associated with this pathology. The first study, conducted with a sample of 480 patients stratified by ethnic origin, identified variants in the *STAG2* (rs151184635) and *RP1-30E17.2* (rs55839915) genes, suggesting a potential genetic component linked to sex. In contrast, the second study, which included 612 patients and employed a multilevel model with multiple ethnic strata, highlighted the genetic variant rs1122449 in the *IRX2* gene as the most significant in relation to aEARR. Additionally, significant risk factors associated with an increased predisposition to aEARR were identified, including male sex, repositioning and repeated adhesion of brackets to the dental surface, and onychophagia. Conversely, the use of brackets with a 0.022” slot was identified as a protective factor against this condition. These results confirm a genetic risk profile that appears to be ethnicity-dependent. Therefore, we conclude that predisposition to aEARR is a pathological process tied to a critical interaction between genetic and clinical factors, with ethnic specificity. This knowledge paves the way for the development of personalized and precision therapeutic strategies in both preventive and therapeutic settings, tailored to the genetic profile of each patient, aimed at optimizing treatments and reducing the risk of secondary pathologies, as is the case with external apical root resorption in orthodontics. The comprehensive analysis of these findings provides an integrated perspective on the diagnostic, ethnic, and genetic factors influencing EARR. In this context, the thesis advocates for a personalized treatment approach, fostering advancements in precision orthodontics and clinical management that not only enhance outcomes but also minimize complications and significantly improve patients’ quality of life.

3. INTRODUCCIÓN

3.1. Contextualización y etiología de la reabsorción radicular apical externa inducida por el tratamiento ortodóncico

La reabsorción radicular apical externa (EARR) se define como un proceso fisiológico o patológico que produce una pérdida permanente e irreversible de la zona apical de la raíz de un diente (1, 2). La **reabsorción fisiológica** se refiere a la reabsorción radicular de los dientes primarios previa a su exfoliación y se desencadena por fuerzas mecánicas del crecimiento y masticación, que activan a los odontoclastos (3). A diferencia de la reabsorción fisiológica, la **reabsorción radicular patológica** afecta tanto a dientes primarios como a permanentes y se desencadena por diversos estímulos, tales como traumatismos, quistes, infecciones y el tratamiento ortodóncico (4, 5). Este último ha sido uno de los más estudiados ya que afecta a aproximadamente el 61% de los pacientes en forma leve (1-2 mm), al 19% en forma moderada, y al 1% en su manifestación más severa (2), e implica la interacción compleja de células y rutas de señalización que median la reabsorción del tejido dentario (3).

El **movimiento dentario ortodóncico** se basa en la aplicación de fuerzas mecánicas a los dientes que desencadenan una adaptación fisiológica del periodonto. Este tejido reacciona a las fuerzas de compresión y tensión mediante una serie de respuestas inflamatorias que alteran el entorno local y afectan al flujo sanguíneo y a la liberación de diversos neurotransmisores, factores de crecimiento y citoquinas (6). Las células del ligamento periodontal (PDL) responden a las cargas mecánicas constantes e intermitentes generando perfiles de expresión génica específicos y adaptaciones celulares que impulsan la remodelación ósea y periodontal, facilitando así el desplazamiento dentario (7).

La **reabsorción radicular** es mediada por células clásticas específicas, como los odontoclastos, que comparten similitudes funcionales con los osteoclastos (1). La respuesta inflamatoria del PDL a estas fuerzas desencadena eventos celulares y moleculares complejos que incluyen remodelación ósea y degradación de la superficie radicular, lo que resulta en una pérdida irreversible de la estructura radicular en casos severos (7, 8). La severidad de la EARR depende de factores genéticos, mecánicos y biológicos, que influyen en la regulación de las moléculas clave involucradas (7, 9).

El **equilibrio entre los macrófagos tipo M1 (proinflamatorios) y M2 (antiinflamatorios)** en el PDL desempeña un papel crucial en la EARR (10). Los macrófagos M1, secretan citocinas como el TNF-α, promueven la inflamación y estimulan la actividad osteoclástica mediante la liberación de óxido nítrico (NO). En contraste, los macrófagos M2, mediados por la interleucina 10 (IL-10) y la arginasa I, tienen un efecto reparador y antiinflamatorio. Bajo fuerzas ortodóncicas prolongadas, se observa un aumento en la proporción M1/M2, lo que intensifica la reabsorción radicular. La plasticidad de estos fenotipos celulares depende del microambiente local y de la interacción con otras moléculas proinflamatorias (1).

Las **vías moleculares clave** en la EARR incluyen la señalización RANKL/RANK/OPG, que regula la diferenciación y activación de los odontoclastos. El RANKL, producido por células del PDL, promueve la formación de células clásticas activas al unirse a su receptor RANK en precursores osteoclásticos (11), mientras que la osteoprotegerina (OPG) actúa como un inhibidor natural al competir con RANKL. Además, la interacción del ATP con el receptor purinérgico P2X7 en respuesta a necrosis celular durante la compresión del PDL, contribuye a la liberación de citocinas inflamatorias como IL-1β, intensificando la reabsorción radicular (12) (*Figura 1*).

La **adhesión de los odontoclastos a la superficie radicular** es mediada por proteínas de la matriz extracelular, como la osteopontina (OPN) y la sialoproteína dentinaria. Estas moléculas facilitan la unión mediante integrinas α/β, promoviendo la polarización celular y la formación de podosomas. Asimismo, la actividad de enzimas lisosomales como las catepsinas y las metaloproteinasas de matriz (MMPs) es esencial para la degradación del tejido mineralizado, completando el proceso de reabsorción radicular (13).

Finalmente, el **cemento dental** es una barrera antirresortiva natural y desempeña un papel protector importante en la raíz. El cemento contiene moléculas y niveles altos de OPG, que inhiben la actividad clástica. Además, las células madre del PDL y la pulpa dental tienen un potencial reparador al modular la expresión de RANKL y OPG (9).

Cuando cesa el estímulo de la fuerza ortodóncica y se retira la presión sobre el ligamento periodontal, el proceso de reabsorción radicular disminuye gradualmente, permitiendo que el tejido periodontal inicie un proceso de reparación y estabilización. Al retirarse la presión constante, se normaliza el flujo sanguíneo y la oxigenación en el PDL, reduciendo el entorno inflamatorio que favorecía la actividad de los odontoclastos.

En ausencia de este estímulo, disminuye la expresión de citoquinas y otros mediadores inflamatorios, como el TNF-α y el RANKL. Esto reduce la formación y la actividad de estas células clásticas y detiene la reabsorción de la raíz. Además, se activa la acción de factores reparadores como la osteoprotegerina (OPG), que bloquea el RANKL, actuando como un freno natural de la reabsorción y favoreciendo la preservación del tejido radicular remanente (8).

En este entorno de menor actividad inflamatoria, los macrófagos M2 aumentan en número y actividad, ayudando a eliminar los restos celulares y a remodelar el tejido, promoviendo una regeneración parcial en el PDL y estabilizando la estructura dental. No obstante, aunque el proceso de reabsorción se detiene, es importante señalar que el tejido radicular perdido no se regenera, ya que el daño es irreversible (9).

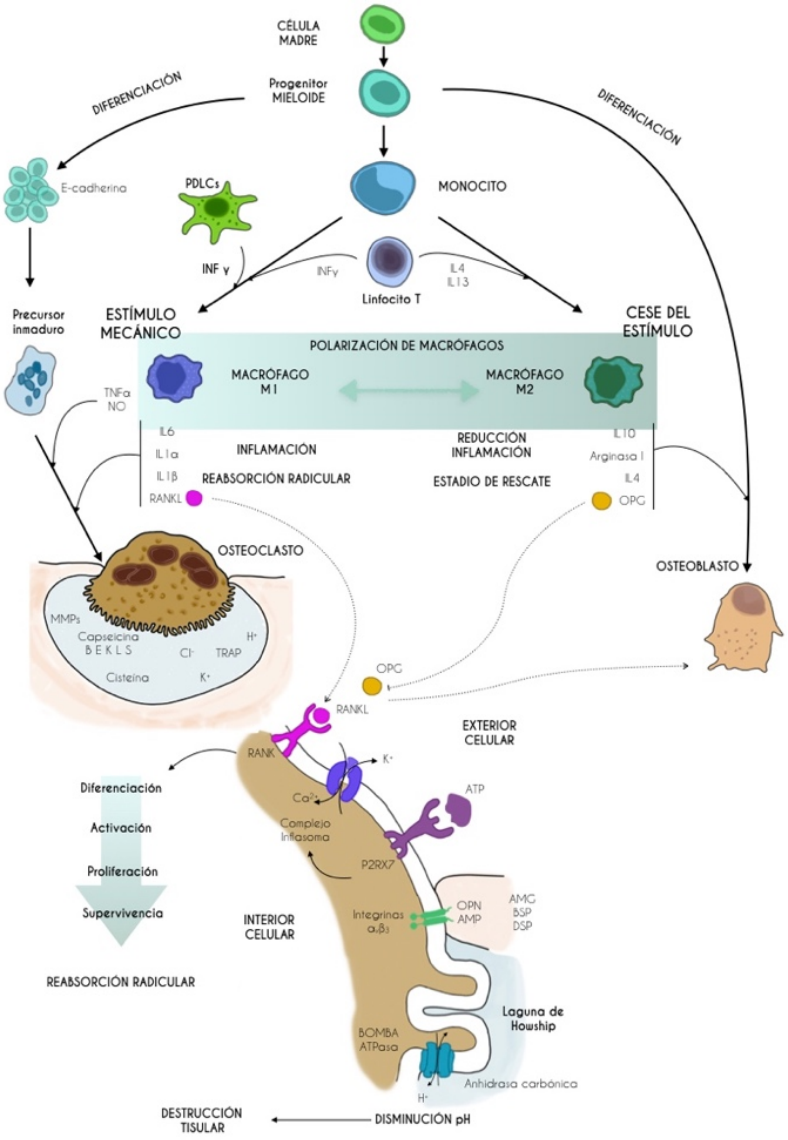


Figura 1: Proceso celular de la reabsorción radicular inducido por fuerzas ortodóncicas

3.2. Diagnóstico de la reabsorción radicular apical externa en ortodoncia

El diagnóstico de la reabsorción radicular apical externa ha sido ampliamente estudiado en la literatura, sin embargo, todavía no existe un consenso sobre el método óptimo para diagnosticar esta patología ni sobre el enfoque más eficaz para medir su severidad (14, 15).

Las **radiografías panorámicas y periapicales** son las herramientas diagnósticas más comúnmente empleadas en la práctica diaria del ortodoncista (16). Aunque su precisión es inferior en comparación con técnicas tridimensionales más avanzadas, como la tomografía computarizada de haz cónico (CBCT) (17), el uso rutinario de éstas en el diagnóstico ortodóncico no está justificado debido a la elevada exposición a radiación que implica (18). Investigaciones recientes han introducido un método novedoso para detectar la reabsorción radicular mediante biomarcadores de la sialofosfoproteína de la dentina (19), en cualquier caso, se requieren estudios adicionales para establecer protocolos que permitan una detección de la reabsorción radicular de manera más fiable y segura para el paciente.

Para la **medición y evaluación de la severidad de la reabsorción radicular** se han empleado diversas metodologías. Estos enfoques van desde técnicas elementales, como la de Mirabella et al. (20) que calcula la diferencia en la longitud de la raíz entre el inicio y el final del tratamiento *(Figura 2).*

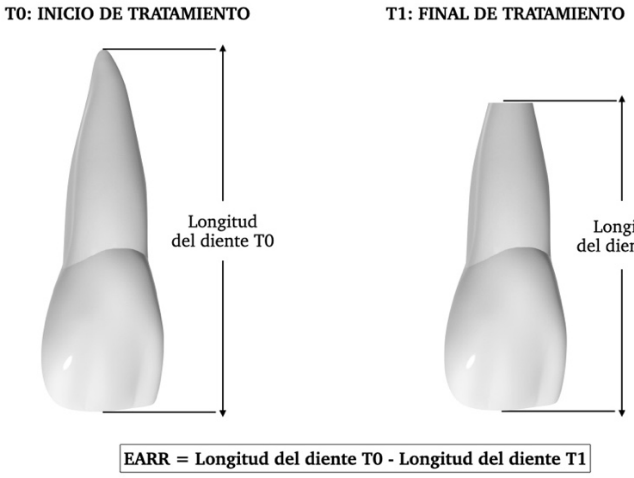


Figura 2. Método de medición de la EARR según Mirabella et al. 1995

Hasta métodos más sofisticados que incorporan factores de corrección en sus mediciones. En 1991, Linge and Linge (21) introdujeron el uso de la línea amelocementaria como punto de referencia para diferenciar la corona de la raíz, asumiendo que la longitud de la corona permanece constante entre las radiografías inicial y final del tratamiento *(Figura 3).* Este método emplea una regla de tres para calcular la diferencia en la longitud de las raíces, utilizando la corona como estructura estable en ambas radiografías. Esta técnica permite una evaluación más precisa de la EARR, al minimizar errores atribuibles a variaciones en la magnificación radiográfica:

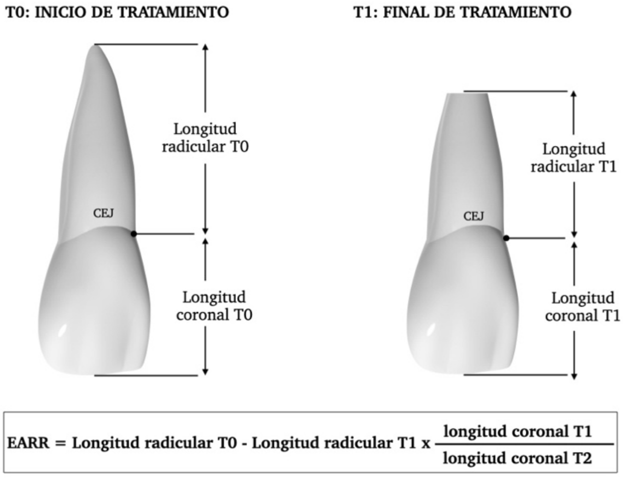


Figura 3: Método de medición de la EARR según Linge and Linge 1991

Posteriormente, Brezniak et al. (22) perfeccionaron el método de Linge y Linge modificando el punto de referencia en la línea amelocementaria calculando un punto medio entre los puntos amelocementarios mesial y distal del diente *(Figura 4),* lo que permite corregir las imprecisiones derivadas de cambios angulares durante la toma de la radiografía.

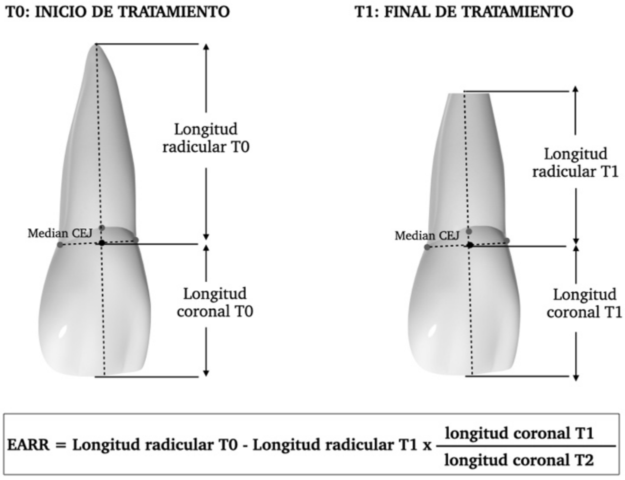
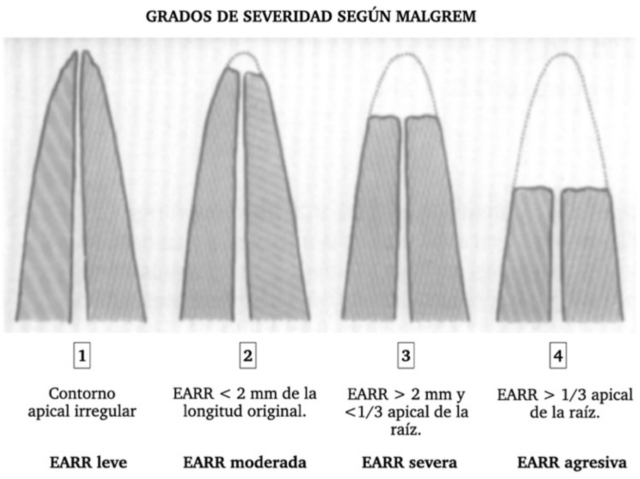


Figura 4: Método de medición de la EARR de Linge and Linge 1991 modificado por Brezniak 2004

Además de los métodos de medición descritos, que son los más comúnmente empleados en la literatura, existen numerosos enfoques adicionales que utilizan distintos factores de corrección, fórmulas alternativas y medidas diferentes a las mencionadas anteriormente (23-25).

En el pasado, las mediciones de la reabsorción radicular apical externa (EARR) se realizaban manualmente utilizando un calibre (21). En la actualidad, la digitalización de las imágenes radiográficas ha permitido la implementación de **métodos de medición informatizados**, como el uso de Adobe Photoshop (23) o software especializados como Dolphin Imaging (25) o el sistema Cranex31 (26). Además, existen sistemas específicos para la medición de la EARR, como el Sistema de Imagen para la Reabsorción Apical (ARIAS) (27). Cuando las mediciones son realizadas en imágenes CBCT, en ocasiones se emplean las mismas técnicas utilizadas en sistemas 2D descritas anteriormente; en otros casos, se recurren a mediciones volumétricas y a softwares específicos (28). La unidad de medida utilizada en las mediciones de la EARR puede ser milímetros (mm), milímetros cúbicos (mm^3), porcentajes (%) o píxeles (“), dependiendo del sistema empleado.

Algunos autores, sin embargo, optan por **métodos de evaluación visual** de la EARR, como el de Malmgrem (29) o Sharpe (30), que proporcionan una clasificación detallada de la severidad de la reabsorción basada en una observación perceptiva del ápice por parte de un evaluador. Estos métodos permiten un enfoque más cualitativo de la medición, clasificando la gravedad de la reabsorción según criterios estandarizados.



Método de medición visual de Malgrem 1982

A pesar de la variedad de técnicas disponibles, **aún no existe un consenso universal sobre el método óptimo para la medición de la EARR**. La elección del método suele depender del criterio, recursos y experiencia de cada clínico, en lugar de estar fundamentada en evidencia científica sólida. Esto subraya la necesidad de realizar estudios comparativos adicionales que evalúen la fiabilidad y precisión de cada metodología, con el fin de establecer un enfoque estándar y respaldado por la investigación.

3.3. Factores de riesgo asociados a la reabsorción radicular apical externa

En el análisis de los factores que influyen en la reabsorción radicular apical externa (EARR) inducida por el tratamiento ortodóntico, es esencial distinguir entre aquellos relacionados directamente con el tratamiento y aquellos asociados con el propio paciente (14).

Diversos estudios han señalado cómo los elementos específicos del plan de tratamiento y la mecánica utilizada pueden influir significativamente en la susceptibilidad del paciente a desarrollar EARR (2). La **duración del tratamiento ortodóncico** se ha identificado como uno de los factores de riesgo más importantes en la aparición de EARR (31), existiendo una correlación positiva entre el tiempo de aplicación de fuerzas ortodóncicas y el grado de reabsorción radicular observado (15, 32-34). No obstante, algunos estudios sugieren resultados no concluyentes sobre esta relación (35).

El enfoque del **tratamiento en dos fases**, que incluye una fase interceptiva temprana seguida de una fase de corrección, se ha asociado con una variación en la incidencia de EARR en comparación con los tratamientos realizados en una sola fase. La aparatología interceptiva puede provocar un estrés adicional en las raíces, aunque la respuesta varía según el tipo de aparato utilizado y el tiempo de intervención en cada fase, y no existe suficiente evidencia para confirmar la asociación (15).

Además, el **tipo de aparatología ortodóncica** parece influir en la reabsorción radicular. Los alineadores transparentes, al ejercer fuerzas intermitentes y ser utilizados en maloclusiones menos complejas, parecen inducir una menor reabsorción en comparación con la aparatología fija multibrackets. Sin embargo, la evidencia aún es controvertida y los resultados entre aparatologías no son comparables, por lo que se requieren más estudios para determinar si los alineadores constituyen una opción menos agresiva en términos de EARR (2, 15, 36-40). La realización de **extracciones** en el tratamiento ortodóncico también se ha asociado con un mayor riesgo de EARR (15, 25, 31, 35), debido a los desplazamientos apicales amplios y la gran magnitud de fuerzas que se requiere. No obstante, algunos estudios no encuentran una relación significativa (41), lo que indica discrepancias en la evidencia actual.

La **complejidad del caso**, determinada por la severidad de la maloclusión y los movimientos requeridos, es un factor crítico en la EARR. Los casos más complejos que requieren movimientos ortodóncicos significativos pueden resultar en una mayor carga sobre el ligamento periodontal y, en consecuencia, en una mayor susceptibilidad a la reabsorción radicular (15). Además, **ciertos movimientos dentarios específicos, como la intrusión, el torque y los desplazamientos apicales**, se han relacionado de manera directa con la reabsorción radicular (7, 15, 35). La intrusión y el torque, en particular, generan fuerzas concentradas en áreas específicas de la raíz, pudiendo aumentar el riesgo de EARR (2, 33, 35, 42-44). Los desplazamientos apicales, al requerir fuerzas de gran magnitud, también incrementan la susceptibilidad del diente a sufrir reabsorción debido a la presión ejercida sobre el ápice radicular (33, 35). Recientemente, nuevas investigaciones han explorado el uso de microtornillos para facilitar la ejecución de estos movimientos y su relación con la EARR, sin llegar a un consenso claro (43-45).

La **disyunción y la distalización de molares** son técnicas que, debido a la magnitud de los movimientos que requieren, pueden presentar un riesgo elevado de EARR. La disyunción maxilar, en particular, implica fuerzas expansivas significativas que podrían afectar a las raíces de los dientes (15), en particular a los premolares (46). Asimismo, **la secuencia de arcos utilizada y la anchura del slot del bracket** (0.018”/0.022”) podrían influir en el tipo y magnitud de las fuerzas aplicadas sobre los dientes. Si bien el uso de arcos rígidos en las fases iniciales del tratamiento se ha asociado con la generación de fuerzas excesivas y mayor susceptibilidad a EARR (2, 47, 48), de momento no se ha encontrado evidencia de que diferentes anchuras del slot incrementen significativamente el riesgo de EARR (49). De manera similar, no existe evidencia sólida de que el **uso de elásticos** sea un factor de riesgo para la EARR. Sin embargo, en investigaciones sobre el **recementado repetido de brackets,** se ha evidenciado que este proceso puede comprometer a la integridad del diente, provocando microfracturas en el esmalte y un aumento del estrés mecánico en las estructuras subyacentes del diente, lo que podría contribuir al proceso de EARR (50).

Por otro lado, los factores de riesgo individuales relacionados con el paciente también juegan un papel fundamental en la EARR. La **edad** del paciente ha sido ampliamente estudiada como un factor relacionado con esta condición (31). En pacientes jóvenes, la mayor capacidad de remodelación ósea y la flexibilidad del ligamento periodontal parecen estar asociadas con una menor incidencia de EARR. En cambio, en pacientes adultos, las características periodontales, la mayor densidad ósea y una menor elasticidad de los tejidos periodontales, pueden aumentar la susceptibilidad a la reabsorción (8, 33). Sin embargo, algunos estudios no han encontrado una relación directa entre la edad y la EARR (15, 51). Además, algunos autores han planteado diferencias en la incidencia de EARR entre **sexos**, sugiriendo una mayor predisposición en mujeres, posiblemente influenciada por factores hormonales. Sin embargo, los resultados disponibles hasta la fecha son inconsistentes y no permiten establecer conclusiones definitivas (31, 35).

La **etnia** del paciente es otro factor que puede influir en la susceptibilidad a la EARR. Diferentes grupos étnicos han mostrado variaciones en la prevalencia de reabsorción radicular, lo que sugiere una posible predisposición genética o biológica a este fenómeno en determinadas poblaciones (52).

La longitud radicular, la proporción corona/raíz y la morfología apical, influyen en la distribución de las fuerzas mecánicas durante el tratamiento ortodóntico (53, 54). Los dientes con raíces más cortas y proporciones corona/raíz reducidas están sujetos a un mayor estrés en una superficie menor, lo que incrementa su susceptibilidad a la reabsorción radicular (55). Estas variaciones, determinadas por la interacción entre factores genéticos y condiciones ambientales, se han propuesto como una posible explicación a las **diferencias étnicas** observadas en la susceptibilidad a la EARR (56). Aunque los hallazgos varían, algunos estudios han sugerido que los caucásicos y los hispanos presentan más reabsorción radicular que otros grupos étnicos, como los asiáticos (52). Sin embargo, debido a las inconsistencias en estos resultados, son necesarios estudios más homogéneos centrados en poblaciones específicas y que empleen protocolos de tratamiento estandarizados para obtener conclusiones más fiables.

La relación esquelética y la maloclusión también podrían influir en la respuesta del ligamento periodontal a las fuerzas ortodóncicas. Pacientes con discrepancias esqueléticas severas o maloclusiones complejas pueden requerir fuerzas más intensas o prolongadas, lo cual incrementaría el riesgo de desarrollar EARR (15, 25, 34), no obstante algunos autores no han encontrado diferencias en la incidencia de EARR entre los distintos tipos de maloclusión (32). Los dientes con **hipoplasia**, así como dientes con **microdoncia,** presentan un área de superficie limitada para la distribución de fuerzas, lo cual los podría hacer más susceptibles a la reabsorción durante el tratamiento ortodóncico, sin embargo, esta relación no ha sido suficientemente estudiada hasta el momento.

Además, se ha sugerido que ciertas condiciones sistémicas, como el **asma y el uso de medicamentos asociados** podrían alterar la respuesta inflamatoria y comprometer la integridad del ligamento periodontal, incrementando el riesgo de EARR (15). Sin embargo, otros estudios no han encontrado evidencia que respalde un impacto significativo de estos medicamentos en el desarrollo de la EARR (32, 57). Hábitos como la **onicofagia** se han vinculado con la reabsorción radicular debido a las fuerzas de estrés constante ejercidas sobre los dientes y las estructuras de soporte, lo que podría exacerbar el riesgo de EARR (58).

En conclusión, la evidencia existente presenta discrepancias significativas y no permite establecer conclusiones definitivas. Esto subraya la importancia de continuar investigando de manera exhaustiva para comprender mejor los mecanismos subyacentes y la interacción de estos factores, incluyendo los genéticos, que se analizarán en el siguiente epígrafe debido a su importancia en esta tesis.

3.4. Marcadores genéticos asociados a la reabsorción radicular apical externa

La genética juega un papel crucial en la regulación de numerosos procesos biológicos y se ha demostrado que las variaciones genéticas pueden influir en la susceptibilidad a diferentes patologías, incluidas las alteraciones dentales como la EARR (59, 60). Un aspecto clave de esta influencia genética son los polimorfismos, que representan variaciones genéticas que ocurren de manera frecuente en una población, con una prevalencia generalmente mayor al 1% (61). Dentro de los polimorfismos, los más comunes son los polimorfismos de nucleótido único (SNP), los cuales consisten en una variación en un solo nucleótido en una posición específica del genoma. Estas pequeñas variaciones pueden alterar la función de proteínas o la expresión de genes, modificando así las respuestas celulares e inflamatorias que contribuyen a procesos como la reabsorción y la función de los odontoclastos, fundamentales en el desarrollo de la EARR (13). Se ha estimado que entre el 50% y el 60% de la variabilidad observada en la susceptibilidad a la EARR podría atribuirse a factores genéticos (62). Del mismo modo, se ha reportado una heredabilidad del 76% para los incisivos centrales maxilares (63), lo cual subraya la relevancia de los factores hereditarios en la susceptibilidad individual a la EARR y sugiere que la predisposición genética juega un papel fundamental en la variabilidad observada entre pacientes. Por otro lado, una revisión sistemática reciente ha señalado que ciertas enfermedades genéticas, como la osteólisis expansiva familiar y el síndrome de Hajdu-Cheney, están asociadas con una actividad osteoclástica aumentada. Esta hiperactividad osteoclástica se ha relacionado tanto con alteraciones óseas sistémicas como con efectos localizados, incluida la reabsorción radicular. Estos hallazgos destacan la importancia de las predisposiciones genéticas en la patogénesis de la reabsorción radicular y abren nuevas líneas de investigación sobre los mecanismos moleculares implicados (59).

Varios genes han sido objeto de estudio en el análisis de la susceptibilidad genética a la EARR, mostrando tanto predisposición como un efecto protector en distintos casos. Estos genes desempeñan roles clave en el proceso de reabsorción radicular al actuar como mediadores inflamatorios, reguladores de la actividad osteoclástica y moduladores de la remodelación ósea y del ligamento periodontal (64).

Los genes de la familia de las interleucinas (IL) están involucrados en la regulación de la inflamación y la respuesta inmune, contribuyendo al ambiente infamatorio asociado con la EARR. Polimorfismos en **IL1A** (rs1800587), han mostrado asociación con un mayor riesgo de EARR (25, 65), así como en I**L1B** (rs1143634) (66-68) y en **IL6** (rs1800796) (69). Adicionalmente el polimorfismo en **IL1RN** (rs419598), puede desempeñar un papel protector o activador frente a la EARR dependiendo del alelo presente y de su influencia en la actividad reguladora de la inflamación (68, 70).

Por otro lado, genes como OPG, RANKL y TNFRSF11B participan directamente en la vía de señalización RANK/RANKL/OPG, fundamental para la diferenciación y actividad de los osteoclastos, células responsables de la reabsorción ósea. Polimorfismos en **OPG** (rs3102724) se han asociado a un efecto protector de la EARR, ya que regula la actividad osteoclástica (71, 72). Por el contrario, polimorfismos en **RANKL** (rs12455775) y

TNFRSF11B (rs3102724, rs2875845, rs1032128, and rs3102728) (71) están asociados a un mayor riesgo de EARR, al promover la activación de osteoclastos y la reabsorción ósea. Asimismo, polimorfismos en los genes implicados en la mediación de señales intracelulares y la activación de rutas inflamatorias como **P2RX7** (rs208294 y rs1718119) (12, 73, 74), **SPP1** (rs9138 and rs11730582) (13, 26) e **IRAK1** (rs1059703) (27), también se han visto asociados a la predisposición a EARR. El receptor de vitamina D, codificado por el gen **VDR**, está implicado en el metabolismo óseo y la homeostasis del calcio. Polimorfismos en VDR *(*rs731236) han sido asociados con la susceptibilidad a EARR (75).

En menor número de estudios, se han identificado otros genes implicados en la homeostasis ósea y la regeneración del ligamento periodontal, como WNT3A, SFRP2, LRP6 y LRP1 (26, 72). Otros polimorfismos en el gen CASP1 (rs530537), en ACTN3 (rs678397) y en TSC2 (rs1051771) también han sido identificados como posibles contribuyentes al fenotipo de EARR y se sugiere su inclusión como SNPs candidatos en futuros estudios (25, 34). También se ha identificado por primera vez el haplotipo CCA de rs17525809, rs208294, rs1718119 en una mayor frecuencia para EARR agresiva (72).

Todas estas asociaciones se han visto en **controversia**, ya que son también muchos los estudios que no logran encontrar relación entre los diferentes polimorfismos y la susceptibilidad a EARR (8, 11, 25, 26, 31, 34, 59, 64, 72). Esto resalta la complejidad en la que múltiples genes y vías de señalización influyen en la predisposición y el desarrollo de la reabsorción radicular. Además, las diferencias metodológicas en el diagnóstico de la EARR entre los estudios dificultan las comparaciones directas y limitan la generalización de los resultados. Por ello, es fundamental profundizar en el estudio de la genética de la EARR mediante metodologías estandarizadas que permitan una mejor comprensión de los factores implicados y una mayor precisión en la identificación de pacientes en riesgo.

4. JUSTIFICACIÓN

La reabsorción radicular apical externa (EARR) inducida por el tratamiento ortodóncico representa una de las mayores barreras para avanzar hacia una ortodoncia verdaderamente segura y personalizada. Su carácter irreversible y su variabilidad en severidad entre los pacientes ponen de relieve la necesidad de explorar en profundidad tanto su diagnóstico, como los factores que influyen en su desarrollo. Actualmente, los métodos de detección no siempre son precisos, los factores de riesgo no están completamente definidos y, en particular, el componente genético permanece en gran medida desconocido. Esta tesis aborda estas áreas críticas con el objetivo de sentar las bases para una ortodoncia preventiva y adaptada a cada individuo.

La complejidad en el diagnóstico y la medición de la EARR plantea un desafío significativo para interpretar adecuadamente la mejor evidencia científica disponible en ortodoncia. Aunque existe consenso sobre su definición, no se han establecido criterios homogéneos que definan claramente los grados de severidad, ni umbrales que permitan categorizar la condición como moderada o agresiva. Además, los métodos para medir la reabsorción radicular son variados, lo que dificulta la comparación de resultados entre estudios y limita el desarrollo de guías clínicas estandarizadas. La incertidumbre respecto a cómo los procesos diagnósticos influyen en la identificación de factores de riesgo asociados, subraya importantes lagunas de conocimiento en este campo. Actualmente, el diagnóstico de la EARR depende de técnicas radiográficas como las radiografías panorámicas y periapicales, los métodos más comúnmente utilizados en la práctica clínica diaria. Establecer métodos diagnósticos precisos y estandarizados es esencial para mejorar la detección temprana de la EARR y su seguimiento en tratamientos ortodóncicos. Esto permite identificar a los pacientes de riesgo de manera más efectiva, promoviendo el desarrollo de protocolos clínicos comparables y contribuyendo a una ortodoncia más segura y fundamentada en la evidencia.

Explorar exhaustivamente todos los factores implicados en la EARR, tanto del paciente como del tratamiento, es crucial para entender y anticipar el riesgo, proporcionando una visión integral y permitiendo desarrollar estrategias de intervención más eficaces y seguras, personalizando los tratamientos ortodóncicos de acuerdo con las particularidades de cada paciente, y promoviendo así un enfoque más seguro y adaptado a sus necesidades específicas.

En este contexto, la susceptibilidad a la EARR no es homogénea en la población; distintos estudios sugieren que ciertos grupos étnicos pueden presentar un mayor o menor riesgo de desarrollar reabsorción radicular en respuesta a las fuerzas ortodóncicas. Esta variabilidad podría estar influenciada tanto por factores genéticos como por características biológicas y ambientales inherentes a cada grupo. El estudio de la variabilidad étnica en la EARR facilita la identificación de grupos de alto riesgo y abre la puerta al desarrollo de intervenciones más adecuadas y menos invasivas para cada paciente. Este enfoque inclusivo es esencial para una ortodoncia de precisión que considere la diversidad de la población. Investigar la relación entre la etnicidad y la susceptibilidad a la reabsorción radicular permitirá a los ortodoncistas adaptar sus estrategias de tratamiento, anticipando y reduciendo el riesgo de EARR en pacientes de diferentes orígenes étnicos.

Adicionalmente, la genética se presenta como un factor clave en la predisposición individual a la EARR. Estudios recientes en genética han identificado ciertos polimorfismos que podrían influir en la susceptibilidad de los pacientes a la reabsorción radicular, sugiriendo que algunos individuos poseen una mayor predisposición genética a esta complicación. La mayoría de estos estudios concluyen que es fundamental contar con muestras de mayor tamaño, emplear criterios diagnósticos consensuados y fiables para la EARR, y llevar a cabo investigaciones adicionales que permitan alcanzar conclusiones definitivas. La identificación de estos factores genéticos no solo es fundamental para entender la EARR en su totalidad, sino que también ofrece la posibilidad de implementar tratamientos ortodóncicos personalizados, donde las decisiones clínicas se basen en el perfil genético del paciente, facilitando la adopción de medidas preventivas y la optimización de las técnicas de tratamiento para minimizar este riesgo. La aplicación de estudios de asociación de genoma completo (GWAS) en la investigación de la EARR representa un avance significativo hacia la ortodoncia de precisión, donde el tratamiento se adapte a las características genéticas de cada individuo. La posibilidad de implementar pruebas genéticas previas al tratamiento podría revolucionar la práctica ortodóncica, permitiendo una detección temprana de los pacientes susceptibles a la EARR y optimizando los resultados a largo plazo.

5. HIPÓTESIS Y OBJETIVOS

El presente trabajo plantea las siguientes hipótesis a contrastar:

1. Existe falta de unificación, estandarización y consenso en la literatura sobre los métodos diagnósticos y de medición de la reabsorción radicular apical externa (EARR). Esto genera discrepancias significativas en los resultados obtenidos y dificulta la comparabilidad entre estudios. Esta variabilidad en los enfoques de diagnóstico, especialmente entre las radiografías panorámicas y periapicales, afecta a la precisión en la evaluación de la EARR y su relación con factores predisponentes. [Capítulo 1, *Paper* #1].
2. Las diferencias morfológicas en la estructura dental y apical, así como la proporción corona/raíz, varían entre los diferentes grupos étnicos, lo que influye significativamente en la susceptibilidad a la reabsorción radicular apical externa (EARR). [Capítulo 2, *Paper* #2].
3. Existe una asociación significativa entre variantes genéticas específicas localizadas a lo largo del genoma, incluyendo los cromosomas sexuales X e Y, y el desarrollo de una reabsorción radicular apical externa agresiva (aEARR). [Capítulo 3, *Paper* #3].
4. Existen variantes genéticas específicas asociadas positiva o negativamente con la predisposición a desarrollar reabsorción radicular apical externa agresiva (aEARR) durante el tratamiento ortodóntico. Además, factores clínicos y diagnósticos del ámbito ortodóncico influyen significativamente en la severidad y progresión de este proceso patológico. [Capítulo 3, *Paper* #4].

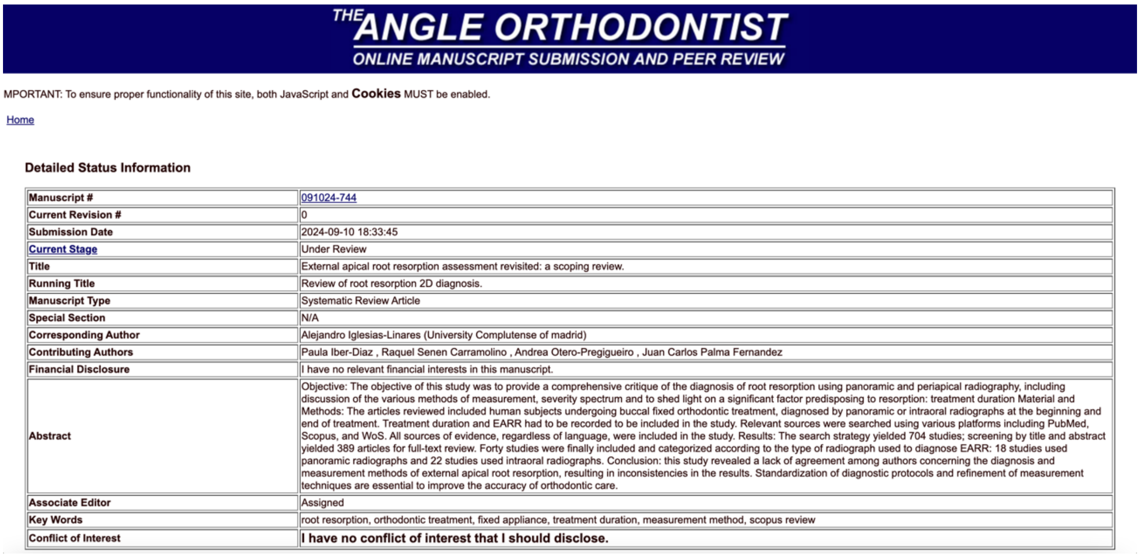
Para contrastar las hipótesis formuladas, se marcan los siguientes objetivos a alcanzar con este trabajo de Tesis Doctoral:

1. Proporcionar una revisión exhaustiva del diagnóstico de reabsorción radicular apical externa realizado mediante radiografías panorámicas y periapicales, los dos métodos más comúnmente utilizados en la práctica clínica. Y de esta forma, mejorar la comprensión y orientar la práctica clínica mediante una evaluación crítica de los métodos de medición empleados, el grado de severidad y la influencia de la duración del tratamiento como factor predisponente a la EARR. [Capítulo 1, *Paper* #1].
2. Analizar cómo las diferencias étnicas influyen en la susceptibilidad a la reabsorción radicular apical externa (EARR) y de esta forma mejorar el desarrollo de planes de tratamiento ortodóncico más personalizados y efectivos, optimizando los resultados para los pacientes. [Capítulo 2, *Paper* #2].
3. Realizar el primer estudio de asociación de genoma completo, llevando a cabo un análisis sobre múltiples loci y genes putativos localizados en los cromosomas somáticos 2, 4, 8, 12, 18, y en los cromosomas sexuales X e Y, potencialmente implicados en la reabsorción radicular apical externa (aEARR). [Capítulo 3, *Paper* #3].
4. Determinar mediante un estudio a genoma completo (GWAS), la asociación entre variantes genéticas específicas y la predisposición a la reabsorción radicular apical externa agresiva (aEARR) con o sin influencia del estrato étnico. Cómo objetivo secundario, se pretende identificar qué factores clínicos potenciales influyen de modo determinante durante el tratamiento ortodóntico. [Capítulo 3, *Paper* #4].

6. CAPÍTULO 1

Métodos diagnósticos y de medición de la reabsorción radicular apical externa

Paper #1. Iber-Diaz P, Senen-Carramolino R, Otero-Pregigueiro A, Palma-Fernandez JC, Iglesias-Linares A. (2024) External apical root resorption assessment revisited: a scoping review. [Under review]. *The Angle Orthodontist.*



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Manuscript #	091024-744
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Corresponding Author	Alejandro Iglesias-Linares (University Complutense of madrid)
Contributing Authors	Paula Iber-Diaz , Raquel Senen Carramolino , Andrea Otero-Pregigueiro , Juan Carlos Palma Fernandez
Financial Disclosure	I have no relevant financial interests in this manuscript.
Abstract	Objective: The objective of this study was to provide a comprehensive critique of the diagnosis of root resorption using panoramic and periapical radiography, including discussion of the various methods of measurement, severity spectrum and to shed light on a significant factor predisposing to resorption: treatment duration Material and Methods: The articles reviewed included human subjects undergoing buccal fixed orthodontic treatment, diagnosed by panoramic or intraoral radiographs at the beginning and end of treatment. Treatment duration and EARR had to be recorded to be included in the study. Relevant sources were searched using various platforms including PubMed, Scopus, and WoS. All sources of evidence, regardless of language, were included in the study. Results: The search strategy yielded 704 studies; screening by title and abstract yielded 389 articles for full-text review. Forty studies were finally included and categorized according to the type of radiograph used to diagnose EARR: 18 studies used panoramic radiographs and 22 studies used intraoral radiographs. Conclusion: this study revealed a lack of agreement among authors concerning the diagnosis and measurement methods of external apical root resorption, resulting in inconsistencies in the results. Standardization of diagnostic protocols and refinement of measurement techniques are essential to improve the accuracy of orthodontic care.
Associate Editor	Assigned
Key Words	root resorption, orthodontic treatment, fixed appliance, treatment duration, measurement method, scopus review
Conflict of Interest	**I have no conflict of interest that I should disclose.**

RESUMEN:

El primer capítulo se centra en revisar de manera crítica la literatura existente sobre los métodos radiográficos empleados para diagnosticar la EARR durante los tratamientos ortodóncicos con aparatología fija, abarcando la variabilidad en las técnicas de medición y los niveles de severidad de EARR. A lo largo de la revisión, se analizaron estudios que utilizaron radiografías panorámicas y periapicales, los cuales son los métodos más frecuentemente empleados para la evaluación diagnóstica en la práctica clínica. Asimismo, se discute la influencia de diversos factores relacionados con el paciente y el tratamiento que pueden predisponer a la aparición de EARR.

Los resultados obtenidos en esta revisión revelan una falta de consenso entre los autores en cuanto a los métodos diagnósticos y las técnicas de medición de la EARR. La revisión también identifica la necesidad urgente de estandarizar los protocolos de diagnóstico y refinar las técnicas de medición para mejorar la precisión y la consistencia en el manejo de la EARR en la práctica clínica ortodóncica. Los estudios evaluados muestran que la duración del tratamiento es un factor que puede influir significativamente en el desarrollo de la EARR, aunque con resultados contradictorios.

Este trabajo destaca la importancia de establecer criterios uniformes que permitan una evaluación más precisa y comparable de la EARR, lo cual contribuirá a mejorar el diagnóstico, la planificación del tratamiento y, en última instancia, los resultados clínicos en ortodoncia.

Paper #1. External apical root resorption assessment revisited: a scoping review.

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External apical root resorption assessment revisited: a scoping review.

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ABSTRACT

Objective: The objective of this study was to provide a comprehensive critique of the diagnosis of root resorption using panoramic and periapical radiography, including discussion of the various methods of measurement, severity spectrum and to shed light on a significant factor predisposing to resorption: treatment duration

Material and Methods: The articles reviewed included human subjects undergoing buccal fixed orthodontic treatment, diagnosed by panoramic or intraoral radiographs at the beginning and end of treatment. Treatment duration and EARR had to be recorded to be included in the study. Relevant sources were searched using various platforms including PubMed, Scopus, and WoS. All sources of evidence, regardless of language, were included in the study.

Results: The search strategy yielded 704 studies; screening by title and abstract yielded 389 articles for full-text review. Forty studies were finally included and categorized according to the type of radiograph used to diagnose EARR: 18 studies used panoramic radiographs and 22 studies used intraoral radiographs.

Conclusion: this study revealed a lack of agreement among authors concerning the diagnosis and measurement methods of external apical root resorption, resulting in inconsistencies in the results. Standardization of diagnostic protocols and refinement of measurement techniques are essential to improve the accuracy of orthodontic care.

INTRODUCTION

In daily orthodontic practice, root resorption continues to be a complex significant challenge [1]. Over the years, a good deal of research has been devoted to this topic [2-4] of critical medical and legal importance in orthodontics. Nevertheless, there is still no consensus on the optimal diagnostic method for this pathology or on the most effective approach to measure its severity [5].

The orthodontics literature has explored various methods of assessing root resorption, ranging from traditional diagnostic radiography to three-dimensional methods using cone-beam computed tomography (CBCT)[6]. Nevertheless, lateral panoramic and periapical radiographs are still the types most commonly used for orthodontic diagnostics in everyday clinical practice [7,8].

In a historical review of the literature, a variety of measurement methodologies have been used to diagnose and assess the severity of root resorption, ranging from elementary techniques [9], to more sophisticated methods involving complicated mathematical formulae [10], or even software analysis[11]. Other methods[12] [13] provide a nuanced classification of the severity of EARR, based on a perceptual assessment of the apex by an observer. These measurement methods are important in determining and classifying the extent of EARR in the individual patient, as they elucidate the specific risk factors for, as well as those that protect against, the occurrence, severity, and development of EARR.
A number of patient-related factors, such as age [6], malocclusion [14], and genetic predisposition [15], have been associated with EARR, with some degree of conflicting results. Treatment duration has been identified in several meta-analyses as a significant treatment-related predisposing factor associated with EARR occurrence and severity [6,16].
The complexities of diagnosing and measuring external apical root resorption (EARR) present a significant challenge in terms of making sense of the best scientific evidence currently available in orthodontics. The uncertainty regarding the influence of EARR diagnostic processes on associated risk factors predictors, highlights critical knowledge gaps. The aim of this paper is to provide a comprehensive review of EARR diagnoses made using panoramic and periapical radiographs, the two methods most commonly used in clinical practice. Secondary aims include enhancing understanding and informing clinical practice by critically evaluating the measurement methods used, the severity spectrum, and the influence of different approaches on the association with treatment duration.

MATERIAL AND METHODS

Protocols and guidelines

This review adhered to the guidelines outlined in the JBI Evidence Synthesis Template and Manual (Peters et al., 2020), as well as the PRISMA extension designed for scoping reviews (Tricco et al.[17]).

Review questions

A participant, concept, context (PCC) question was proposed to select eligible studies: *"Is the literature consistent regarding the radiographic diagnostic method, measurement, and severity scale of external apical root resorption during orthodontic treatment with fixed appliances?"*

Inclusion criteria

Participants: The review included human subjects undergoing their first orthodontic treatment with fixed appliances, without adjunct therapies, systemic/periodontal diseases, or medications affecting oral health. Exclusion criteria included a history of dental trauma or root canal treatment of upper incisors. EARR diagnosis in at least one upper incisor was required, measured by periapical/panoramic radiographs, with recorded treatment duration.

Concept: The study critically evaluates diagnostic methods using panoramic and periapical radiographs, examining severity and treatment duration as a key factor for resorption.

Context: Global evidence from various sources was accepted.

Types of sources of evidence: In vitro studies, animal research, histological studies, reviews, and opinion pieces were excluded. No restrictions on study duration or language were imposed, as long as the participant, concept, and context criteria were met.

Search strategy

The search strategy focused on the concept of root resorption in the context of orthodontic treatment. Relevant sources were searched using different platforms, including PubMed, Scopus and WoS. Selected studies that met the inclusion criteria were then carefully reviewed. The authors of the primary literature were contacted directly when necessary and all sources of evidence, regardless of language, were considered for inclusion. Details of the search methodology, including relevant keywords and index terms tailored to each of the databases consulted are provided in *Appendix 1*.

Source of evidence screening and selection

The screening process was divided into four steps: (1) removal of duplicates, [18] review of title, (3) review of abstract, and (4) review of full-text. This was carried out independently by two researchers (PID and AOP). Disagreements were resolved by consensus or by a third reviewer (AIL).

Data extraction

Data extraction was conducted by one reviewer (PID) and verified by a second (AOP). Data were divided into two categories: panoramic versus periapical radiographs, further subdivided by measurement method and whether EARR results were reported in percentages (%) or millimeters (mm). The variables recorded are described in *Table 1 and 2.*

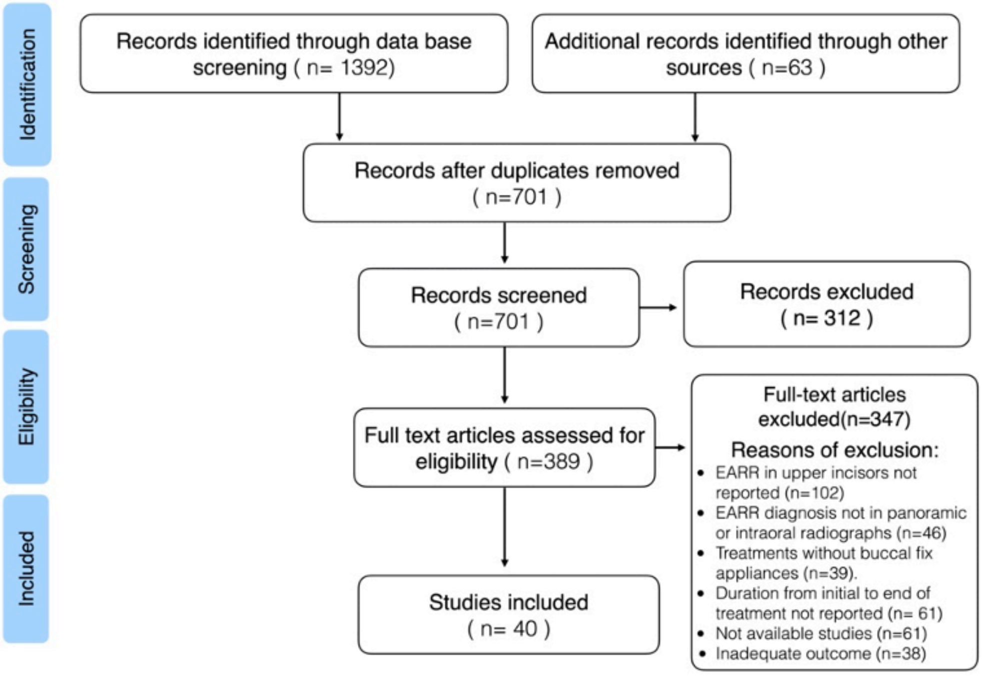
Analysis and presentation of results

A table was created to extract data from the different studies. In cases where only one group met the inclusion criteria, the article was included presenting only the calculated sample size and data for that group.

RESULTS

Search results.

Our search strategy yielded 704 studies, of which 3 were duplicates. Following the PCC criteria, screening by title and abstract yielded 389 articles for full-text review and 40 studies were finally included in this review [3,4,8,9,11,13,19-52]. The PRISMA flowchart in *Figure 1* illustrates the process. Reasons for exclusion are summarized and described in *Appendix 2.*



Flow diagram for the scoping review process adapted from the PRISMA statement by Moher and colleagues (2009)

Characteristics of included studies: type of radiographic method and sample size

After screening and checking that the studies met the inclusion criteria, 40 were finally selected. All the included publications were categorized according to type of radiograph used to diagnose EARR: 18 studies used panoramic radiographs [4,8,11,38-52] and 22 studies used intraoral radiographs [3,9,13,19-37]. The studies included according to radiographic method, panoramic radiographs and intraoral radiographs, are synthesized and summarized in *Tables 1* and *2,* respectively.

For sample size, an important distinction was made between the number of patients analyzed in the studies and the number of roots measured to determine EARR. The mean number of patients treated in the studies was 130. For the number of roots measured, the study by Pamukçu et al. [47] examined only 30 roots, whereas Linge and Linge [3] studied 1656.

Table 1

Characteristics of included studies that used panoramic radiography for the diagnosis of EARR.

Author Year	**Country**	**Sample size (upper incisors roots measured)**	**Sample Size (patients)**	**Treatment duration (months)**	**EARR measurement method**	**EARR measurement programm**
Alexander **1996** **[37]**	Stony Brook, N.Y. EEUU	112	56	Range 22-27	Sharpe et al (Scale)	Visual Sharpe
Nigul et al. **2006** **[45]**	Tartu, Estonia	281	75	Range 4-38	Linge and Linge (mm)	NR
Pandis et al. **2008** **[47]**	Athens, Greece.	384	96	26.43 ± 6.29	Correction facctor: *Assessment of the magnification of the maxillary incisors in panoramic radiographs was performed by inclusion of a graded tip of a periodontal probe in the panoramic radiographs. The metal tip was temporarily bonded between the maxillary central incisors from the incisal edge and upward.* (mm)	With a 3-times magnifying glass and a finetip digital caliper with accuracy up to .01 mm (Mitutoyo Digi- matic NTD12-6□C, Mitutoyo, Kanagawa, Japan) inter- faced with an Excel spreadsheet (Microsoft, Redmond, Wash).
Iglesias-Linares et al. **2012** **[40]**	Seville, Spain	54	54	31.1 ± 6.4	Linge and Linge modified by Brezniak (mm)	Nemoceph Dental Studio
Llamas-Carreras et al. **2012** **[44]**	Seville, Spain	38	38	24.0 ± 12.0	Correction factor: *in order to allow intra-patient standardization, root resorptions in the root filled tooth (RRE) and contralateral tooth with vital pulp (RRV) were calculated. Then, the proportion of root resorption (PRR) for each patient was calculated as follows: PRR = RRE / RRV.* (mm)	Adobe Photoshop CS® software
Rakhshan et al. **2012** **[49]**	Tehran, Iran	451	132	28.54 ± 9.71	Linge and Linge (mm)	NR
Linhartova et al. **2013** **[43]**	Brno, Czech Republic	424	106	34.5 ± 15.6	Linge and Linge modified by Brezniak (mm)	NR
Iglesias-Linares et al. **2014** **[41]**	Seville, Spain	87	87	27.5 ± 8.3	Linge and Linge modified by Brezniak (mm)	NR
Jacobs et al. **2014** **[42]**	Mainz, Germany	852	213	19.8 ± 5.2	Linge and Linge modified by Brezniak (%)	NR
Pereira et al. **2014** **[48]**	Coimbra, Portugal	195	195	36 ± 10	Linge and Linge modified by Brezniak (%)	ARIAS: Apical Resorption Image Analysis System, Porto, Portugal. MATLAB version 7.12.0.635 (R2011a, MathWorks company, Natick, MA, USA).
Savoldi et al. **2015** **[50]**	Brescia, Italy)	156	93	25.2	Correction factor: *For each film, the length of the mesio-distal diameter of the crown of the mandibular right first molar was measured in pixels, and then all the measurements were converted using this value as the specific unit for each patient.*	Adobe Photoshop CS6®
Yi et al. **2018** **[51]**	Chengdu, China	11: 40 12:40 21:40 22:40 TOTAL: 160	40	20.83 ± 5.29	Linge and Linge modified by Brezniak (%)	Marosis Enterprise PACS; Infinitt Healthcare, Seoul, Korea
Qin et al. **2019** **[4]**	Wenzhou, Zhejiang, China	98	98	21.17 ± 5.13	Linge and Linge modified by Brezniak (mm)	NR
Pamukçu et al. **2020** **[46]**	Ankara, Turkey	11:30 12:30 21:30 22:30 TOTAL: 120	30	26.87 ± 7.67	Fritz et al Gay et al 2017 (%)	ImageJ (US National Institutes of Health, Bethesda, MD, USA).
Ciurla et al. **2021** **[39]**	Lublin, Poland	404	101	31.1 ± 6.4	Linge and Linge modified by Brezniak (%)	Planmeca Romexis Viewer software
Lee et al. **2022** **[11]**	Seoul, South Korea	118	118	33.6 ± 8.3	Linge and Linge modified by Brezniak (mm)	Cranex31 system (Soredex, Helsinki, Finland) ZeTTA PACS Viewer
Baghaei et al. **2023** **[38]**	Birmingham, AL, USA	780	195	19.4 ± 6.4	Correction factor: *We utilized the ruler on the cephalometric radiograph to measure the molar crown width and transferred that measurement to calibrate the panoramic radiographs.* (%)	Dolphin Imaging software (Chatsworth, CA, USA).
Kaya et al **2023** **[8]**	Bahcelievler -Ankara, Türkiye	412	103	27.21 ± 7.63	Malgrem	Visual Malgrem

Table 1

Characteristics of included studies that used panoramic radiography for the diagnosis of EARR.

Author Year	Rx Parameters	EARR Definition and severity	Mean EARR founded	% Severity of EARR	Prevalence of EARR
Alexander **1996** **[37]**	The same Ritter Midwest Panoral (Des Plaines, Ill.) and Gendex GX900 (Gendex, Milwaukee, Wis.) machines were used to obtain the panoramic and occlusal films.	Sharpe	Mean Score scale of Sharpe: Maxilla central incisors: 0.28±0.07 Lateral central incisors: 0.37±0.9 TOTAL: 0.32 ± 0.5	NR	22.00%
Nigul et al. **2006** **[45]**	Different x-ray machines were used to obtain the panoramic radiographs; the position of the patient was not standardized.	Sharpe	12: 1.57±1.2mm 11: 1.61±1.27mm 21: 1.48±1.05mm 22: 1.35±1.02mm TOTAL: 1.50±1.13mm	Scale of Sharpe: 0: 12% 1: 64% 2: 14.6% 3: 2.6% Missing: 6.6.%	88.00%
Pandis et al. **2008** **[47]**	NR	NR	11: 1.29 ± 1.03mm. 12: 1.44 ± 1.11mm. 21: 1.17 ± 1.11mm. 22: 1.29 ± 1.21mm. TOTAL: 1.29 ± 1.11mm	NR	NR
Iglesias-Linares et al. **2012** **[40]**	NR	EARR >2mm No EARR<2mm	EARR Group: 3.12±0.71mm No EARR Group: 1.06±0.5mm TOTAL: 2.09±0.6mm	NR	EARR>2mm: (n:25) 46% EARR<2mm: (n:29) 54%
Llamas-Carreras et al. **2012** **[44]**	Promax®, Planmeca, class 1, type B, 80 KHz, Planmeca, Helsinki, Finland	NR	1.1 ± 1.0mm	NR	NR
Rakhshan et al. **2012** **[49]**	Same panoramic unit (Odontorama PC, Trophy Radiologie, Marne La Valle, France) + Other unknown units	EARR= Root pre-treatment – (Root post-treatment × Correction Factor). The percentage changes in the root lengths were calculated using Copeland and Green's criteria, EARR ≥ 1.20 mm was regarded as clinical EARR.	1,377 ± 1,214 mm	NR	Central incisor: 32,9% Lateral incisor: 58,3%
Linhartova et al. **2013** **[43]**	NR	EARR >2mm No EARR<2mm	EARR Group: 2.31±0.47mm No EARR Group: 0.51±0.5mm TOTAL: 1.41±0.97mm	NR	EARR>2mm: (n:74) 70% EARR<2mm: (n:32) 30%
Iglesias-Linares et al. **2014** **[41]**	NR	EARR >2mm No EARR<2mm	NR	EARR>2mm: (n:37) 42% EARR<2mm: (n:50) 58%	EARR>2mm: (n:37) 42% EARR<2mm: (n:50) 58%
Jacobs et al. **2014** **[42]**	NR	Malgrem	NR	Teeth affected by severe EARR: 12,22:1 (0.2%) 11,21: 1 (0.2%)	NR
Pereira et al. **2014** **[48]**	Both radiographs were performed with the same equipment.	NR	12: 11±0.9 % 11: 10± 0.8% 21: 10±1 % 22: 10±0.8% TOTAL: 10±0.8%	NR	NR
Savoldi et al. **2015** **[50]**	NR	NR	11: 0.3 ± 9.3% 12: 1.4 ± 10.1%. 21: 0.6 ± 9.3%. 22: 1.8 ± 10.9%. TOTAL: 1.02 ± 9.9%	NR	NR
Yi et al. **2018** **[51]**	NR	NR	11,21: 6.80 ± 3.90%. 12,22:7.08 ± 3.86%. TOTAL: 6.94 ± 3.88%	NR	NR
Qin et al. **2019** **[4]**	Radiographs were taken before and after treatment with the same radiographic machine (SIEMENS, SIDEXIS XG, Germany)	Malgrem	11: 0.36 ± 0.19mm. 12: 0.28 ± 0.03mm. 21: 0.36 ± 0.25mm. 22: 0.29 ± 0.09mm. TOTAL: 0.32 ± 0.14	0: 9.18%. 1: 32.65%. 2: 38.77%. 3: 11.22%. 4: 8.16%.	90.82%
Pamukçu et al. **2020** **[46]**	64–66 kVp 6–9mA 10s	Gay et al rRCR ≥ 100% presenting no EARR, rRCR = 90–99% slight EARR, rRCR = 80–90% moderate EARR, rRCR < 80% severe EARR	NR	11,12,21,22: Severe: (n:13) 0.32 11% Moderate: (n: 22) 18% Slight: (n:26) 22% No EARR: (n:59) 49%	11: 53,33%. 12: 46,6%. 21: 46,6%. 22: 56,6%
Ciurla et al. **2021** **[39]**	NR	EARR: 0.90>rRCR<1 No EARR: rRCR > 0.90	TOTAL: rRCR<0.80: 2.97% (12) 0.80<rRCR<0.90: 5.91% (34) 0.90<rRCR<1: 86.88% (351) rRCR<1: 1.73% (7)	TOTAL: rRCR<0.80: 2.97% (12) 0.80<rRCR<0.90: 5.91% (34) 0.90<rRCR<1: 86.88% (351) rRCR<1: 1.73% (7)	TOTAL: rRCR<0.80: 2.97% (12) 0.80<rRCR<0.90: 5.91% (34) 0.90<rRCR<1: 86.88% (351) rRCR<1: 1.73% (7)
Lee et al. **2022** **[11]**	NR	EARR >2mm No EARR<2mm	2.9±2.4mm	EARR>2mm: (n:59) 50% EARR<2mm: (n:59) 50%	EARR>2mm: (n:59) 50% EARR<2mm: (n:59) 50%
Baghaei et al. **2023** **[38]**	NR	EARR was recorded when at least 20% of the root length of at least 1 incisor had been lost with orthodontic treatment.	EARR: 27% (n:53)	NR	27%%
Kaya et al **2023** **[8]**	NR	Malgrem	Degree of root resorpion difference in Malgrem: 12: 1.73±0.79 11:1.59±0.74 21:1.59±0.74 22: 1.72±0.80 TOTAL: 1.65± 0.76	NR	NR

Table 2

Characteristics of included studies that used panoramic radiography for the diagnosis of EARR.

Author Year	Country	Sample size (upper incisors roots measured)	Sample Size (patients)	Treatment duration (months)	EARR measurement method
Sharpe et al. 1987 [13]	NY,US	72	36	37.4	Sharpes Method (Scale)
McFadden et al. 1989 [27]	Göteborg, Sweden	38	38	28.8 ± 7.4	Linge and Linge (mm and %)
Remington et al. 1989 [29]	Washington	11,21: 198 12,22: 191 TOTAL: 389	100	26.4 ± 9.5	Malgrem (Scale)
Linge and Linge 1991 [3]	Skien, Norway	12: 428 teeths 11: 422 teeths 21: 392 teeths 22: 414 teeths TOTAL: 1656	485	12: 10.5±7 11: 12.2± 7.6 21: 12.2 ± 7.5 22: 10.8 ± 7 TOTAL: 11.42 ± 7.2	Linge and Linge (mm)
Mirabella and Artun 1995 [9]	Washington	11,21: 299 12,22: 290 TOTAL: 589	343	24 ± 8.3	Mirabella (mm)
Blake et al. 1995 [20]	Toronto, Canada	252	63	20.8 ± 4.5	Linge and Linge (%)
Lupi et al. 1996 [24]	Illinois, Chicago	11,21: 166 12,22: 161 TOTAL: 327	88	20	Sharpe (Scale)
Taithongchai et al. 1996 [32]	Bangkok and Nonthaburi, Thailand, and St. Louis, Mo.	800	400	20.96 ± 8.35	Linge and Linge (mm)
Reukers et al. 1998 [30]	Nijmegen, Netherlands.	61	61	20.4 ± 5.3	OWN METHOD *To correct for different projection angles that are a consequence of the bisecting-angle technique, the radiographs were digitally processed according to the method as described by Reukers et al.* *After reconstruction of the digital images, the (relative) tooth lengths could be measured as the number of pixels on the screen. The percentage loss of tooth length was calculated as (L1-L2)/Ll• (L1 = tooth length before treatment; L2 = tooth length at the end of fixed appliance therapy).* (%)
Sameshima and Sinclair 2001 [31]	Los Angeles California	867	868	31.4 ± 7.4	Mirabella (mm)
van Loenen et al. 2007 [34]	Ghent, Belgium	11,21: 50 12,22: 49 TOTAL: 99	31	27.6 ± 6	CORRECTION FACTOR *The edge of the bracket, cementoenamel junction, and root apices were marked and used to define crown and root length.* *The ARR ratio was calculated as follows (Figure 1): C1/ C2·R2/R1, where C is the crown length and R the root length at different time points. When a tooth showed no root resorption during the different treatment periods, the ARR ratio was classified as 1.* (mm)
Bellamy et al. 2008 [19]	Seattle, USA.	87	43	28	Apex - CEJ (following the long axis) (mm)
Artun et al. 2009 [18]	Safat, Kuwait.	997	267	24.9 ± 7.3	OWN METHOD *The protocol called for 3 radiographic projections, 1 with the central ray between the 2 central incisors, and 1 with the ray centered at the lateral incisor on either side, made according to a paralleling technique. Then, correction reconstruction and superimposition of the images.* *TL was measured as the distance from the apex tip to the midpoint of either the incisal edge or the line connecting the mesial and distal outlines of ce- mentoenamel junction, depending on the location of the incisal reference points used for reconstruction* (mm)
Liou and Chang 2010 [23]	Taipei, Taiwan	80	20	22.7 ± 5.0	Linge and Linge (mm and %)
Picanço et al. 2011 [28]	Brazil	99	99	43.14 ± 11.1	Apex - CEJ (following the long axis) (mm and %)
Martins et al. 2012 [25]	São Paulo, Bauru, Brazil	224	56	28.69 ± 8.25	Malgrem (Scale)
Zahed et al. 2013 [35]	Shiraz, Irán.	375	127	23.98	Apex - CEJ (following the long axis) (%)
Zawawi and Malki 2014 [36]	Jedda, Saudi Arabia	80	40	21.5 ± 3.39	Linge and Linge (mm)
Maues et al. 2015 [26]	Rio de Janeiro, Brazil	11:121 12:118 21:120 22:118 TOTAL: 477	129	85.79 ± 47.63	Malgrem (Scale)
Chen et al. 2015 [21]	Zhejiang, China	280	70	20.43 ± 3.51	Linge and Linge (mm and %)
Tehranchi et al. 2017 [33]	Fort Lauderdale USA	11:34 12:34 21:34 22:34 TOTAL: 136	34	46.91 ± 20.03	Linge and Linge modified by Brezniak (%)
Kim et al. 2018 [22]	Seoul, South Korea.	11,21: 135 22,12: 135 TOTAL: 270	135	25.7 ± 10.5	OWN METHOD: Apex-CEJ with correction factor *Pretreatment tooth length (R1) was measured along the long axis connecting the midpoint of the incisal edge (M) and the root apex, and the intersection angle (h) between the incisal edge and the long axis was recorded. Posttreatment tooth length (R2) was mea- sured along the long axis originating from M with an intersection angle of h to the incisal edge. The correction factor (CF) was calculated as the ratio of the pretreatment ball height (B1) and the posttreatment ball height (B2) to correct any differences in image magnification or distortion between pretreatment and posttreatment radiographs. ARR was calculated as follows:CF=B1/B2. ARR = R1 - (R2xCF)* (mm)

Table 2

Characteristics of included studies that used panoramic radiography for the diagnosis of EARR.

Author Year	EARR measurement programm	Rx Parameters
Sharpe et al. **1987** **[13]**	Visual Sharpe	NR
McFadden et al. **1989** **[27]**	The measurements were done with good illumination and electronic measuring calipers! (resolution to 0.01 mm and accuracy to 0.03 mm).	NR
Remington et al. **1989** **[29]**	NR	NR
Linge and Linge **1991** **[3]**	Directly from the radiographs.	NR
Mirabella and Artun **1995** **[9]**	The radiographs were projected onto a screen at approximately x 7 magnification. Linear measurements were made with a transparent ruler to the nearest fourteen-hundredth of a millimeter, the nearest whole millimeter on the magnified image.	NR
Blake et al. **1995** **[20]**	A specialized measuring instrument called the Comparitor (Edmund Scientific Co., Barrington, N.J.) was used. It has a 6 x eyepiece with a 20 mm scale etched onto the viewing surface.	NR
Lupi et al. **1996** **[24]**	Visual Sharpe	NR
Taithongchai et al. **1996** **[32]**	Each pretreatment and posttreatment periapical radiograph of the maxillary central incisors was enlarged 10 times by projection and traced.	NR
Reukers et al. **1998** **[30]**	NR	NR
Sameshima and Sinclair **2001** **[31]**	The full-mouth periapical films were scanned then viewed at double magnification on a large color monitor with 0.25 dot pitch fineness. Root length was mea-sured on the scanned images from the apex to the mid- point of the right and left cementoenamel junctions with Sigma Scan (SPSS Scientific, Chicago, Ill).	NR
van Loenen et al. **2007** **[34]**	Jasc® Paint Shop Pro 7TM (Eden Prairie, USA).	The radiographs were developed, magnified (×3), and digitized using Agfa ScanWise 1.2.0.5® (Mortsel, Belgium).
Bellamy et al. **2008** **[19]**	ImageJ	NR
Artun et al. **2009** **[18]**	Emago software, recording the number of pixels between landmark pairs	NR
Liou and Chang **2010** **[23]**	ImageJ	NR
Picanço et al. **2011** **[28]**	Dolphin Imaging Premium 10.5 (Dolphin Imaging & Management Solutions, Chatsworth, USA)	NR
Martins et al. **2012** **[25]**	Scanned with the Sprint Scan 35 Plus scanner (version 2.7.2; Polaroid, Cambridge, Massachusetts, USA), with a resolution of 675 dpi at a scale of 1:1. The images were analysed with Photoshop software (version 6.0; Adobe System, San Jose, California, USA) at 300 per cent enlargement, without image quality loss.	NR
Zahed et al. **2013** **[35]**	Photoshop S3	NR
Zawawi and Malki **2014** **[36]**	Sirona Sidexis software	NR
Maues et al. **2015** **[26]**	Visual. Malgrem x-ray viewer with standard light intensity, equipped with a 5-x magnification loop (Cristófoli Equipamen- tos de Biossegurança Ltda., Campo Mourão, Paraná, Brazil)	NR
Chen et al. **2015** **[21]**	The measures were performed to the nearest 0.01 mm, using the image analysis system (SIEMENS, SIDEXIS XG, Germany)	NR
Tehranchi et al. **2017** **[33]**	The digital radiographs were then visualized and analyzed through Photoshop CS (Adobe Systems Inc., San Jose, CA, USA). To measure the distances, a proprietary tool was developed on MATLAB's image processing toolbox (MATLAB 7.14 2012a, Mathworks Inc., MA, USA).	Digital X-ray unit (MinRay, SOREDEX, Helsinki, Finland – (10 kvp at 8 mA, 0.1 S); Processor: ACETON PSPIX, France) at the same distance and using the same exposure settings (70–85 kVp at 10 Ma)
Kim et al. **2018** **[22]**	Image J 1.43u software program (Wayne Rasband, National Institutes of Health, Bethesda, Md) with B1 height defined as 4 mm.	NR

Table 2

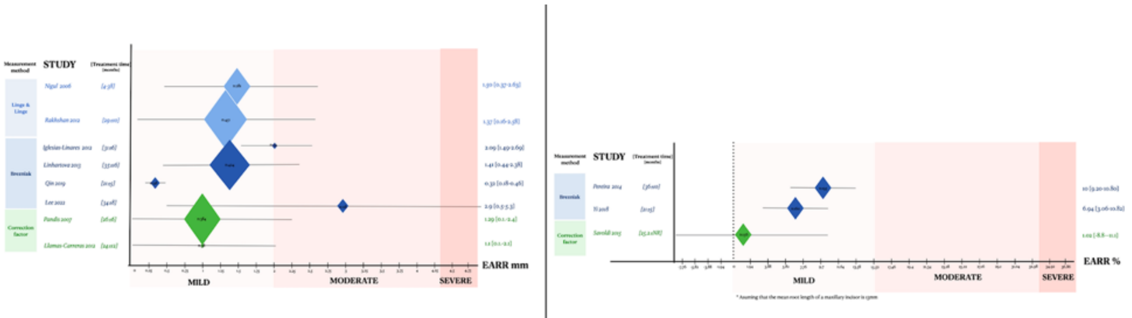
Characteristics of included studies that used panoramic radiography for the diagnosis of EARR.

Author Year	EARR Definition and severity	Mean EARR founded	% Severity of EARR	Prevalence of EARR
Sharpe et al. 1987 [13]	Severity according to Sharpes Method (Scale)	Sharpes Method Media Score 0,72 ± 0,165	NR	41.65%
McFadden et al. 1989 [27]	NR	1.84mm / 13.2%	NR	NR
Remington et al. 1989 [29]	Malgrem	TOTAL: (n:389) 0:15 (4%) 1:19 (5%) 2:255 (65%) 3:90 (23%) 4: 10 (3%)	TOTAL: (n:389) 0:15 (4%) 1:19 (5%) 2:255 (65%) 3:90 (23%) 4: 10 (3%)	11,21 (n:198): 0: 1% 1,2,3,4: 99% 12,22 (n:191): 0: 6% 1,2,3,4: 94%
Linge and Linge 1991 [3]	RR > 0mm	12: 1 ± 1 mm 11: 0.8 ± 0.9 mm 21: 0.7 ± 0.9 mm 22:1 ± 1 mm TOTAL: 0.87 ± 1 mm	Eighty patients (16.5%) had root shortenings >2.5 mm for one or more max- illary incisors.	n: 1656 teeths 89% RR > 0mmm
Mirabella and Artun 1995 [9]	Severity from -1mm to 9 mm RR	11,21 (n:299): 1.12±1.39mm 12,22 (n:290): 1.23±1.25mm TOTAL: 1.22±1.32mm	NR	NR
Blake et al. 1995 [20]	Root resorption was defined as any reduction in the radiographic length of the maxillary and mandibular incisor teeth from the tip of the incisal edge to the apex of the root.	12,22: 12.52% ± 8.88 11,21: 8.35% ± 7.68 TOTAL: 10.47%±8.28	NR	NR
Lupi et al. 1996 [24]	Sharpes Method	TOTAL (n:327) Pretreatment/Posttreatment: 0:259/78 (79%/23%) 1:60/158 (18%/48%) 2:7/79 (2%/24%) 3:1/12 (0.5%/3.5%)	TOTAL (n:327) Pretreatment/Posttreatment: 0:259/78 (79%/23%) 1:60/158 (18%/48%) 2:7/79 (2%/24%) 3:1/12 (0.5%/3.5%)	TOTAL (n:327) Pretreatment/Posttreatment: 0:259/78 (79%/23%) 1:60/158 (18%/48%) 2:7/79 (2%/24%) 3:1/12 (0.5%/3.5%)
Taithongchai et al. 1996 [32]	NR	21: 1.96±1.33mm 11: 2.11±1.37mm TOTAL: 2.04±1.22mm	2% of the patients in the present sample lost more than 5 mm.	NR
Reukers et al. 1998 [30]	NR	7.8% ± 6.9	NR	65.00%
Sameshima and Sinclair 2001 [31]	NR	11,21: 1.17 ± 1.14 mm 12,22: 1.43 ± 1.27 mm	NR	NR
van Loenen et al. 2007 [34]	NR	11,21: 0.89±0.08mm 12,22: 0.85±0.10mm TOTAL: 0.87±0.09 mm	NR	11,21: 70% 12,22: 76%
Bellamy et al. 2008 [19]	NR	TOTAL: 0.43±0.14mm	NR	NR
Artun et al. 2009 [18]	NR	12: 1.24±1.26mm 11:1.01±1.05mm 21:0.88±1.17mm 22:0.95±1.17mm TOTAL: 1.10±1.02mm	>5mm:	<2mm: 76.8% >2mm: 16.5% >3mm: 5.2% >4mm:1.5%
Liou and Chang 2010 [23]	Shortening of the original root length. Apical root resorption (mm) = C1/C2 (R1 – R2); apical root resorption (%) = C1/C2 (R1 – R2)/R1.	12: 2.1 ± 1.4 mm (14.4 ± 7.3 %) 11: 2.1 ± 1.5 mm (13.6 ± 7.6%) 21: 2.1 ± 1.3 mm (13.4 ± 7.3%) 22: 2.3 ± 1.7 mm (13.6 ± 7.6%) TOTAL: 2.15 ± 1.4 mm	NR	NR
Picanço et al. 2011 [28]	Malgrem	2.06±0.94mm (0.17%±0.09)	The sample is divided by severity	The sample is divided by severity
Martins et al. 2012 [25]	Malgrem	Malgrem: 0: 0% (n:0) 1: 28% (n:62) 2: 43% (n:97) 3: 26% (n:58) 4: 3% (n:7)	Malgrem: 0: 0% (n:0) 1: 28% (n:62) 2: 43% (n:97) 3: 26% (n:58) 4: 3% (n:7)	100.00%
Zahed et al. 2013 [35]	NR	11, 21: 16,76% 12,22: 16,99%	NR	NR
Zawawi and Malki 2014 [36]	Malgrem	11,21: 0.99 ± 0.11 mm	No RR: 32.5% Mild (<2mm): 56.2% Moderate (>2 and >1/3 of root): 8.8% Severe (>1/3 of root): 2.5%.	67.5%
Maues et al. 2015 [26]	Malgrem	TOTAL: (n:457) 0: 98 (20%) 1: 73 (15%) 2: 191 (44%) 3: 69 (14%) 4: 26 (7%)	TOTAL: (n:457) 0: 98 (20%) 1: 73 (15%) 2: 191 (44%) 3: 69 (14%) 4: 26 (7%)	TOTAL: (n:457) 0: 98 (20%) 1: 73 (15%) 2: 191 (44%) 3: 69 (14%) 4: 26 (7%)
Chen et al. 2015 [21]	Malgrem	11,21: 0.4±0.3mm (3.37%). 12,22: 0.25±0.4mm (2.30%) TOTAL: 0.32±0.35mm (2.83%)	NR	100.00%
Tehranchi et al. 2017 [33]	NR	21:0.91 ± 0.08 mm/8.49 ± 8.77% 11: 0.88 ± 0.09 mm/11.88 ± 9.37% 22: 0.85 ± 0.11 mm/14.04 ± 11.40 % 12: 0.87 ± 0.10 mm/12.45 ± 10.10% TOTAL: 0.87 ± 0.10/ 11.71 ± 9.91%	NR	NR
Kim et al. 2018 [22]	NR	11,21: 1.09 ± 0.49 mm 12,22: 1.08 ± 0.52 mm TOTAL: 1.08 ± 0.50mm	NR	NR

External apical root resorption (EARR): definition and threshold values, type of results, and severity.

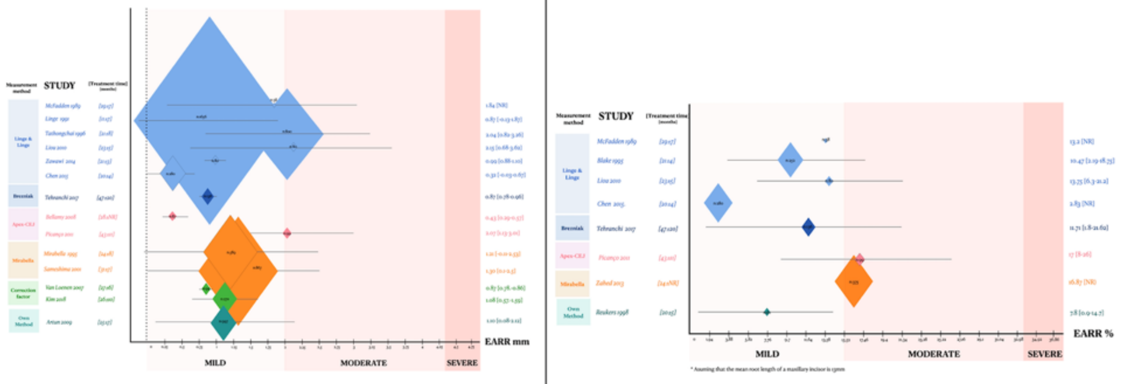
There is a bewildering array of techniques and perspectives for diagnosing and estimating external apical root resorption (EARR). While some researchers set the threshold for defining EARR at 2 mm[41] of apical root loss, others[50], considered even minimal losses of 1.20 mm as significant. Surprisingly, there were even those who suggested the onset of EARR as early as 0.1 mm [39,47]. The adoption by some investigators of Malmgrem's [12] or Sharpe's [13] severity scales introduced further complexity into the assessment of EARR.

The EARR results compiled in *Fig. 2* provide interesting data and insights into the diagnosis of EARR. The findings are mostly presented in millimeters (mm) or percentages (%), and vary from study to study. More particularly, Chen et al. [22] and Qin et al.[4] report minimal mean EARR values (0.32±0.14 mm), which contrast with Lee et al. [11] notably higher measurement (2.9±2.4 mm). In terms of percentage measurements, Picanco et al. [35] reported the lowest EARR (0.17%±0.09), whereas Zahed et al. [36] reported the highest (16.87%).



a) Studies on panoramic radiographs describing the results of EARR in millimeters.

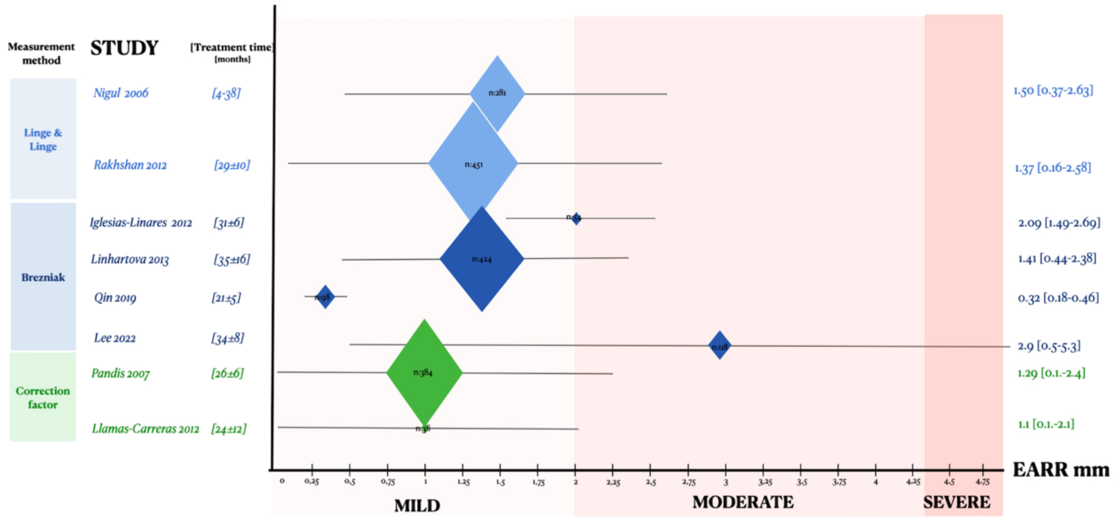
b) Studies on panoramic radiographs describing the results of EARR in percentages.



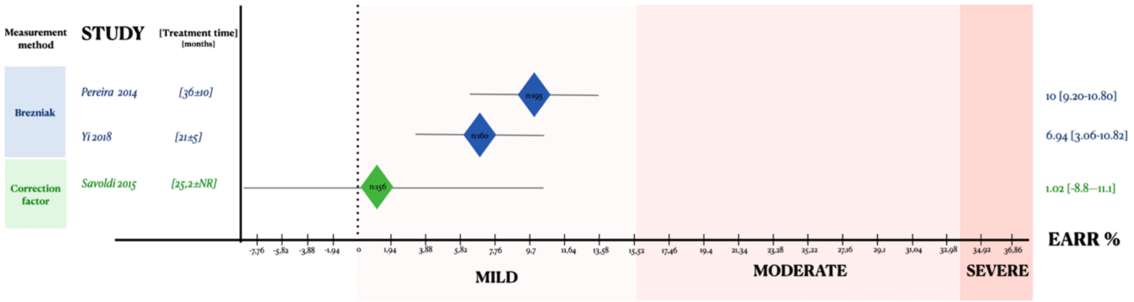
c) Studies on intraoral radiographs describing the results of EARR in millimeters.

d) Studies on intraoral radiographs describing the results of EARR in percentages.

Fig. 2*: Studies on panoramic and periodical radiographs describing the results of EARR in millimeters and percentages.*

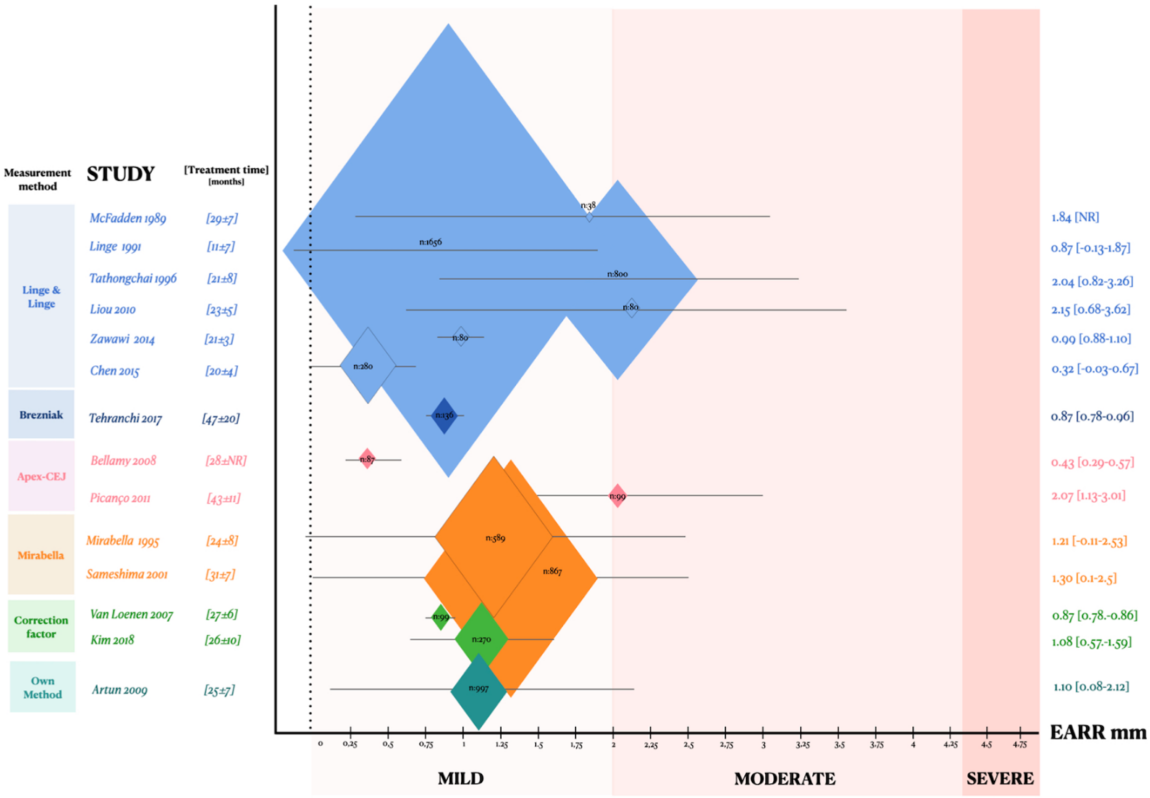


a) Studies on panoramic radiographs describing the results of EARR in millimeters.

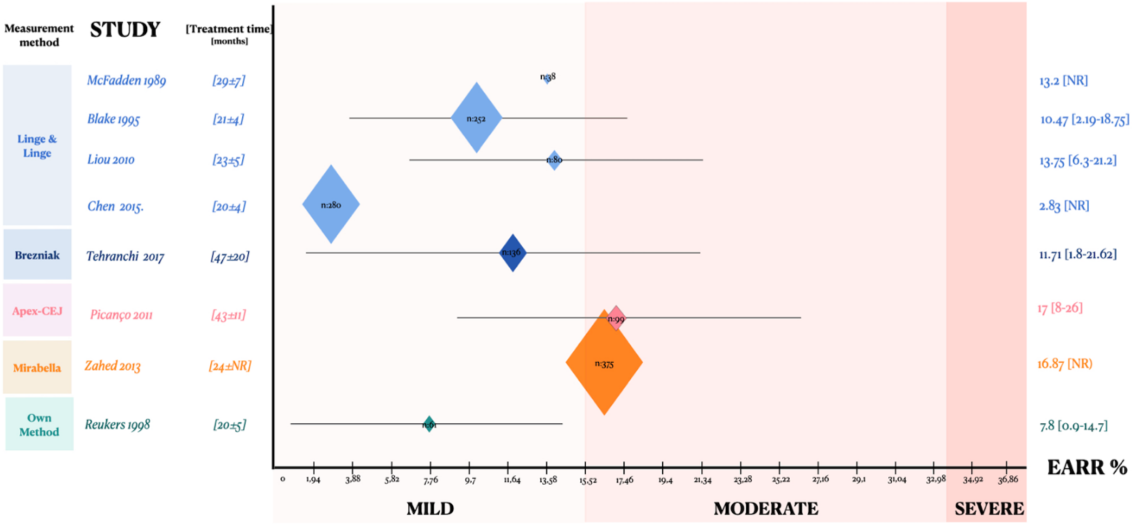


* Asuming that the mean root length of a maxillary incisor is 13mm

b) Studies on panoramic radiographs describing the results of EARR in percentages.



c) *Studies on intraoral radiographs describing the results of EARR in millimeters.*



* Asuming that the mean root length of a maxillary incisor is 13mm

d) *Studies on intraoral radiographs describing the results of EARR in percentages.*

Measurement method, software analysis, and radiographic parameters. The most commonly used method for measuring EARR is that developed by Linge's method modified by Brezniak [10], employed in 25%, followed by the original Linge and Linge method[3], utilized in 22.5% of the studies. The prevalence of each measurement method's usage is presented in *Appendix 3*.

The software or direct measurement methods used for assessments are varied and numerous, and additionally, other authors have developed their own software for measurement. (*Table 1* and *Table 2*). Only two studies [34,47] have reported the exact radiographic parameters utilized for X-ray imaging; however, these parameters vary considerably depending on the type of radiograph taken.

Duration of treatment as a cofounding factor associated with EARR.

The mean duration of orthodontic treatment across the 40 studies was 28.10 months. Treatment duration was found to be positively correlate with the amount of EARR in 13 studies and not associated in 5. The remaining studies did not analyze treatment duration as a factor related to the amount of EARR. However, there was not found relation between the measurement method and the studies affirming a correlation of treatment time with EARR.

DISCUSSION

After reviewing the results of this study, it is clear from the literature that there are no common criteria for the diagnosis of EARR. Some authors[23] used periapical radiographs for diagnosis, whereas others [52] used panoramic radiography. Different methods for EARR measurement were reported, some using the incisal edge as a correction factor [23], and others [52] using the modified Linge and Linge method by Brezniak [10]. The assessment method also varies, with some expressing results in millimeters (mm), and others reporting EARR in percentages (%). With all the differences in diagnostic technique, measurement method and units, it can be concluded that the discrepancies in the scientific literature come from many different sources.

It should be noted that the study designs of the included studies did not differ but there were notable differences in sample size between the studies, and a wide range of teeth examined in each study, this could compromise the representativeness of the results. To mitigate these differences and strive for consistency, the present study only included studies that focused on maxillary incisors, which are not only the ones most commonly used in studies focusing on EARR, but are also more prone to EARR [14].

The use of cone-beam computed tomography (CBCT) is currently not a feasible alternative to routine panoramic, mainly for economic and ethical reasons, particularly in young patients [8,53]. Consequently, panoramic and periapical radiographs are still the preferred imaging modalities for diagnostic purposes and for routine assessment of root resorption [8]. Nevertheless, some concerns have been raised regarding the potential overestimation of root loss by panoramic radiographs compared to intraoral radiographs, with some studies suggesting an overestimation of 20% or more [32]. In the present study, panoramic radiographs showed an average EARR of 1.49 mm compared to 1.22 mm in periapical radiographs. It should be noted however that this difference can be attributed to possible overestimation by panoramic radiographs or to inherent variability in the results of the studies themselves. This can be attributed in part to proclination of the upper incisors, which was not verified by measuring proclination on cephalometric radiographs. Hence, it would be of great interest to include incisor proclination as a factor in the formula for calculating resorption when using panoramic radiographs to diagnose EARR [54].

The use of different measurement methods in different studies is a significant source of bias when interpreting the results. However, it should be noted that the cementoenamel junction can be used as a reliable landmark for correction and some authors [32] concluded that its identification is often challenging in radiographs. Despite this, a surprising 62% of the studies analyzed in this review used it to diagnose EARR. Some authors[23] used a metal ball cemented to the tooth, whereas others [39] used the mesiodistal size of the molar. The lack of consistency in current literature highlights the urgent need for consensus on EARR diagnostic methods, measurement techniques, and interpretation criteria. Variability across studies leads to discrepancies in prevalence rates and treatment outcomes, hindering the creation of standardized clinical protocols. Collaborative efforts among researchers, clinicians, and professional organizations are essential to develop universally accepted guidelines for imaging modalities, measurement standardization, and clear criteria for EARR severity. Such consensus will enhance research comparability, improve diagnosis accuracy, and facilitate better monitoring and management of EARR in orthodontics and dentistry.

CONCLUSIONS

The findings revealed a notable lack of consensus among the authors reviewed regarding the diagnosis and methods of measurement of root resorption (EARR). Furthermore, the variability of the measurement techniques suggests that no single method consistently provides comparable results. This inconsistency poses a major challenge to the accurate assessment and management of root resorption in clinical practice. Resolving this issue is critical to improving diagnostic accuracy, treatment planning, and ultimately patient outcomes in orthodontic care. Future research should focus on standardizing diagnostic protocols and refining measurement techniques to establish a more uniform approach to assessing root resorption using 2D radiography. The present scoping review allowed us to conclude that:

1. The orthodontic literature describes a wide range of assessment methods for EARR, ranging from traditional 2D and 3D radiographic techniques to innovative approaches such as biomarker-based detection or aided by artificial intelligence.
2. There is considerable variability in the methods used to measure EARR across studies, with some utilizing correction factors and others relying on perceptual assessment to classify severity.
3. Patient- and treatment-related factors such as treatment duration are inconsistently associated with the development of EARR.
4. Standardization of diagnostic criteria, measurement methods, and severity classification is essential to increase reliability and comparability between studies and to improve clinical management of EARR.

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FIGURES LEGENDS

Fig. 1*: Flow diagram for the scoping review process adapted from the PRISMA statement by Moher and colleagues (2009)*

Fig. 2*: Studies on panoramic and intraoral radiographies describing the results of EARR in millimeters or percentages.*

Conflict of interest:

None. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethic statement:

This study was conducted under a pre-doctoral teaching and research contract (0103/AYU/003) at Complutense University of Madrid.

Appendix 1
Search Strategy

Last search date: 15.03.2024

PUBMED = 626

((((((((((("root resorption"[MeSH Terms]) OR ("root resorption"[Title/Abstract])) OR ("tooth resorption"[Title/Abstract])) OR ("root shortening"[Title/Abstract])) OR (EARR[Title/Abstract])) OR ("root resorptions"[Title/Abstract])) OR (OIRR[Title/Abstract])) OR (root resorptions[MeSH Terms])) OR ("apical root resorption"[Title/Abstract])) OR ("external apical root resorption"[Title/Abstract])) OR (aggresive external apical root resorption[Title/Abstract])) AND ((((((((((((((("orthodontic treatment"[Title/Abstract]) OR ("orthodontic appliances"[Title/Abstract])) OR ("orthodontic appliance"[Title/Abstract])) OR ("fixed appliances"[Title/Abstract])) OR (bracket[Title/Abstract])) OR ("orthodontic forces"[Title/Abstract])) OR ("orthodontic movement"[Title/Abstract])) OR ("tooth movement"[Title/Abstract])) OR ("teeth movement"[Title/Abstract])) OR (braces[Title/Abstract])) OR (bracket, orthodontic[MeSH Terms])) OR (brackets, orthodontic[MeSH Terms])) OR (orthodontic bracket[MeSH Terms])) OR (orthodontic brackets[MeSH Terms])) OR (braces[MeSH Terms])) OR (Post-Orthodontic[Title/Abstract])) NOT ((((((((((((((((((((((((((((((((((((review, systematic[MeSH Terms])) OR (in vitro[MeSH Terms])) OR (dog[MeSH Terms])) OR (animal[Title/Abstract])) OR (beagle[Title/Abstract])) OR (mice[Title/Abstract])) OR (review[Title/Abstract])) OR (rats[Title/Abstract])) OR ("deciduous dentition"[Title/Abstract])) OR ("deciduous teeth"[Title/Abstract])) OR ("in vitro"[Title/Abstract])) OR (animal model[MeSH Terms])) OR (animal model, experimental[MeSH Terms])) OR (impacted teeth[MeSH Terms])) OR (impacted tooth[MeSH Terms])) OR (impacted[Title/Abstract])) OR (trauma[Title/Abstract])) OR (avulsion[Title/Abstract])) OR (retreatment[Title/Abstract])) OR (avulsed[Title/Abstract])) OR (syndrome[Title/Abstract])) OR ("cleft palate"[Title/Abstract])) OR (rat[Title/Abstract])) OR ("primary tooth"[Title/Abstract])) OR (dogs[Title/Abstract])) OR ("root fracture"[Title/Abstract])) OR (cleft[Title/Abstract])) OR (ectopic[Title/Abstract])) OR (autotransplant*[Title/Abstract])) OR ("third molar"[Title/Abstract])) OR (ankylosed[Title/Abstract])) OR (histologic*[Title/Abstract])) OR ("orthognathic surgery"[Title/Abstract])) OR (supernumerary[Title/Abstract])) OR (trauma*[Title/Abstract])) OR (expansion[Title/Abstract]))

SCOPUS = 750

TITLE-ABS ("root resorption" OR "root resorption" OR "tooth resorption" OR "root shortening" OR "EARR" OR "root resorptions" OR "OIRR" OR "root resorptions" OR "apical root resorption" OR "external apical root resorption" OR "aggresive external apical root resorption") AND TITLE-ABS ("orthodontic treatment" OR "orthodontic appliances" OR "orthodontic appliance" OR "fixed appliances" OR "bracket" OR "orthodontic forces" OR "orthodontic movement" OR "tooth movement" OR "teeth movement" OR "braces" OR "orthodontic bracket" OR "orthodontic brackets" OR "Post-Orthodontic") AND NOT TITLE-ABS ("systematic review" OR "review" OR "in vitro" OR "dog" OR "dogs" OR "animal" OR "beagle" OR "mice" OR "rats" OR "rats" OR "deciduous dentition" OR "deciduous teeth" OR "animal model" OR "impacted teeth" OR "impacted tooth" OR "impacted" OR "trauma" OR "avulsion" OR "retreatment" OR "avulsed" OR "syndrome" OR "cleft palate" OR "primary tooth" OR "root fracture" OR "cleft" OR "ectopic" OR "autotransplant" OR "third molar" OR "ankylosed" OR "histologic" OR "orthognathic surgery" OR "supernumerary" OR "trauma" OR "expansion")

WOS = 16

(((((((((AB=(EARR)) OR AB=(root resorption)) OR AB=(tooth resorption)) OR AB=(root shortening)) OR AB=(root resorptions)) OR AB=(OIRR)) OR AB=(root resorptions)) OR AB=(apical root resorption)) OR AB=(external apical root resorption)) AND ((((((((((((AB=(orthodontic appliances)) OR AB=(orthodontic treatment)) OR AB=(orthodontic appliance)) OR AB=(fixed appliances)) AND AB=(bracket)) OR AB=(orthodontic forces)) OR AB=(orthodontic movement)) OR AB=(tooth movement)) OR AB=(teeth movement)) OR AB=(braces)) OR AB=(orthodontic bracket)) AND AB=(orthodontic brackets)) OR AB=(Post-Orthodontic) NOT (((((((((((((((((((((((((((((((((AB=(in vitro)) NOT AB=(dog)) NOT AB=(dogs)) NOT AB=(beagle)) NOT AB=(animal)) NOT AB=(mice)) NOT AB=(rat)) NOT AB=(rats)) NOT AB=(deciduous dentition)) NOT AB=(deciduous teeth)) NOT AB=(animal model)) NOT AB=(impacted teeth)) NOT AB=(impacted tooth)) NOT AB=(impacted)) NOT AB=(trauma)) NOT AB=(avulsion)) NOT AB=(retreatment)) NOT AB=(avulsed)) NOT AB=(syndrome)) NOT AB=(cleft palate)) NOT AB=(primary tooth)) NOT AB=(root fracture)) NOT AB=(cleft)) NOT AB=(cleft)) NOT AB=(ectopic)) NOT AB=(autotransplant)) NOT AB=(third molar)) NOT AB=(ankylosed)) NOT AB=(histologic)) NOT AB=(orthognathic surgery)) NOT AB=(supernumerary)) NOT AB=(trauma)) NOT AB=(expansion)) NOT AB=(systematic review)

Appendix 2.

SOURCES EXCLUDED FOLLOWING FULL-TEXT REVIEW = 347

Title	DOI	Reasons for exclusion
External apical root resorption after orthodontic treatment: Incidence, severity and risk factors	10.34172/joddd.2021.017	Treatment without buccal fix appliances
Prevalence and severity of apical root resorption during orthodontic treatment with clear aligners and fixed appliances: a cone beam computed tomography study	10.1186/s40510-019-0301-1	EARR diagnosis not in panoramic or intraoral radiographs
External apical root resorption after orthodontic treatment: analysis in different chronological periods	10.1590/2177-6709.27.5.e2220100.oar	Treatment without buccal fix appliances
Expression of biological markers in gingival crevicular fluid of teeth with orthodontically induced root resorption	10.1007/s00056-020-00267-x	Duration from inital to end of treatment not reported
External root resorption evaluated by CBCT 3D models superimposition	10.1590/2177-6709.27.2.e2219315.oar	Duration from inital to end of treatment not reported
Tooth resorptions are not hereditary	10.1590/2177-6709.22.4.022-027.oin	Inadequate outcome: Invitro/animal studies or reviews
Genetic and treatment-related risk factors associated with external apical root resorption (EARR) concurrent with orthodontia	10.1111/ocr.12078	Duration from inital to end of treatment not reported
Evaluation of the Effect of Different Bracket Systems on External Apical Root Resorption Using Cone-Beam Computed Tomography	10.5152/TurkJOrthod.2021.20029	EARR in upper incisors not reported
Genetic determinants and postorthodontic external apical root resorption in Czech children	10.1111/odi.12564	EARR in upper incisors not reported
Stainless steel and NiTi torque archwires and apical root resorption	10.1007/s00056-020-00244-4	Duration from inital to end of treatment not reported
Predisposing factors for external apical root resorption associated with orthodontic treatment	10.4041/kjod.2019.49.5.310	Treatment without buccal fix appliances
Effect of micro-osteoperforations on external apical root resorption: A randomized controlled trial	10.4041/kjod.2021.51.2.86	EARR in upper incisors not reported
Bone Density and Dental External Apical Root Resorption	10.1007/s11914-016-0340-1	Inadequate outcome: Invitro/animal studies or reviews
External root resorption during orthodontic treatment in root-filled teeth and contralateral teeth with vital pulp: A clinical study of contributing factors	10.1016/j.ajodo.2015.06.027	EARR in upper incisors not reported
Negligible tooth resorptions after anterior open bite treatment using skeletal anchorage with miniplates	10.1590/2177-6709.25.4.016-022.oin	Inadequate outcome: Invitro/animal studies or reviews
External apical root resorption and vectors of orthodontic tooth movement	10.1016/j.ajodo.2019.10.017	EARR diagnosis not in panoramic or intraoral radiographs
GENETIC FACTORS IN EXTERNAL APICAL ROOT RESORPTION AND ORTHODONTIC TREATMENT	10.1177/154411130401500205	Inadequate outcome: Invitro/animal studies or reviews
Postorthodontic external root resorption in root-filled teeth is influenced by interleukin-1β polymorphism	10.1016/j.joen.2011.12.022	EARR in upper incisors not reported
Factors Associated to Apical Root Resorption after Orthodontic Treatment	10.2174/1874210601812010331	EARR in upper incisors not reported
Association between root resorption and tooth development: A quantitative clinical study	10.1016/j.ajodo.2019.11.011	Treatment without buccal fix appliances
Radiographic and histochemical assessment of root resorption during canine retraction through piezocision site	10.4103/ijdr.IJDR_654_19	Not avaliable
The correlation between external apical root resorption and electric pulp test responses: a prospective clinical trial	10.1590/2177-6709.26.3.e2119389.oar	Duration from inital to end of treatment not reported
Genetic predisposition to external apical root resorption in orthodontic patients: linkage of chromosome-18 marker	10.1177/154405910308200506	EARR in upper incisors not reported

(15 pages)

Appendix 3.

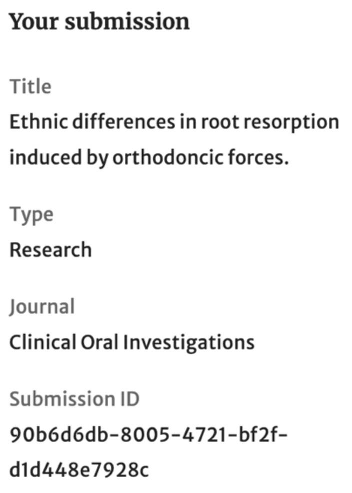
Prevalence of measurement methods used included studies

Measurement Method	Panoramic radiograph	Intraoral radiograph	TOTAL
Linge and Linge modified by Brezniak	9	1	10 (25%)
Linge and Linge	2	7	9 (22,5%)
Correction factor	4	1	5 (12,5%)
Malgrem	1	3	4 (10%)
Sharpe	1	2	3 (7,5%)
Apex-CEJ	0	3	4 (7,5%)
Own Method	0	3	5 (7,5%)
Mirabella	0	2	2 (5%)
Fritz and gay	1	0	1 (4%)

7. CAPÍTULO 2

Diferencias étnicas en la reabsorción radicular inducida por fuerzas ortodóncicas

Paper #2. Iber-Diaz P, Dehesa-Santos A, Senen-Carramolino R, Palma-Fernandez JC, Iglesias-Linares A (2024). Ethnic differences in root resorption induced by orthodontic forces. [Under review]. *Clinical Oral Investigations.*



Your submission

Title
Ethnic differences in root resorption induced by orthodoncic forces.

Type
Research

Journal
Clinical Oral Investigations

Submission ID
90b6d6db-8005-4721-bf2f-d1d448e7928c

RESUMEN:

El segundo capítulo examina en profundidad las variaciones étnicas en la susceptibilidad a la reabsorción radicular apical externa (EARR) y se exploran factores como la morfología del ápice dental, la proporción corona/raíz y la duración del tratamiento, para comprender cómo influyen en la EARR entre distintos grupos étnicos.

La muestra de 200 pacientes, clasificados en tres grupos étnicos (europeos del sur, latinoamericanos y europeos del este), reveló diferencias significativas en la incidencia y severidad de la EARR, especialmente en el incisivo lateral superior. Los resultados mostraron que los pacientes latinoamericanos presentan una menor incidencia de reabsorción leve o moderada en comparación con los pacientes europeos, lo que puede atribuirse a la mayor prevalencia de morfologías apicales puntiagudas en estos pacientes, una característica que podría conferir mayor resistencia frente a las fuerzas ortodóncicas, disminuyendo así el riesgo de reabsorción. Además, los pacientes europeos del sur mostraron una proporción corona/raíz significativamente mayor en comparación con los europeos del este, lo que también podría influir en la susceptibilidad a la EARR.

Otro hallazgo relevante es que, aunque los factores étnicos y morfológicos parecen influir en los casos de reabsorción leve a moderada, no se observaron diferencias significativas entre grupos étnicos en las formas más agresivas de EARR. Esto sugiere que, si bien los factores étnicos pueden afectar la susceptibilidad a niveles moderados de reabsorción, la progresión hacia reabsorciones más severas probablemente esté impulsada por otros factores independientes de la etnia, como la duración del tratamiento ortodóncico, que se identificó como un factor crítico.

En conclusión, este estudio pone de manifiesto la importancia de considerar las variaciones étnicas y morfológicas en la planificación ortodóncica. Las diferencias observadas en la forma del ápice y en la proporción corona/raíz no solo condicionan el riesgo de desarrollar reabsorción radicular, sino que también subrayan la necesidad de adaptar los tratamientos a las particularidades de cada paciente, minimizando complicaciones y optimizando los resultados. Además, estas variaciones morfológicas entre grupos étnicos sugieren que podrían existir fenotipos diferenciados que influyan en la respuesta al tratamiento ortodóncico. Explorar esta dimensión genética ofrecería una oportunidad invaluable para avanzar hacia enfoques de ortodoncia aún más precisos y personalizados.

Paper #2. Ethnic differences in root resorption induced by orthodontic forces.

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Ethnic differences in root resorption induced by orthodoncic forces.

Authors names and affiliations: Paula Iber-Diaz[a,b], Alexandra Dehesa-Santos[a], Raquel Senen-Carramolino[a], Juan Carlos Palma Fernández[a], Alejandro Iglesias-Linares*[a,b]

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[b]BIOCRAN, Craniofacial Biology and Orthodontics Research Group, School of Dentistry, Complutense University of Madrid, Madrid, Spain.

Short title: ethnic differences in orthodontic root resorption

Keywords: root resorption, apical resorption, ethnicity, ethnic variation, orthodontics, orthodontic forces.

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ABSTRACT

Objectives: This study aimed to provide a comprehensive understanding of how ethnic differences influence susceptibility to external apical root resorption (EARR). The findings of this study are intended to inform the development of more personalized and effective orthodontic treatment plans, ultimately enhancing patient outcomes and advancing orthodontic treatment.

Materials and Methods: This retrospective study included 200 patients divided into South European (n=145), Latin American (n=39), and East European (n=16) groups. Panoramic radiographs measured EARR in upper central and lateral incisors using the Malgrem visual method and the modified by Brezniak Linge and Linge method. Crown/root ratio and apical morphology were also analyzed. Statistical tests included t-tests, chi-squared, and ANOVA ($p < 0.05$).

Results: Latin American patients showed a higher prevalence of pointed apex morphology ($p = 0.010$) and lower rates of mild/moderate EARR than South Europeans ($p = 0.041$). Additionally, South Europeans had a higher crown/root ratio (1.46 ± 0.20) than East Europeans (1.33 ± 0.16) for the upper lateral incisor ($p = 0.007$).

Conclusion: Ethnic differences affect the risk of EARR, particularly in the upper lateral incisor. Latin American patients showed a higher prevalence of pointed apex morphology and lower rates of mild/moderate EARR than Europeans. Additionally, treatment duration was linked to an increased risk of severe EARR, although no ethnic differences were observed in the most aggressive forms.

Clinical Relevance: Recognizing ethnic variations in EARR susceptibility can help orthodontists develop risk-based, personalized treatments, potentially reducing adverse effects and improving care across diverse populations.

Key words: root resorption, ethnicity, origins, apical morphology, orthodontics.

INTRODUCTION

Ethnicity, genetics, and demographic factors have become increasingly relevant in the study of medical and orthodontic treatment [1, 2]. These factors shape a patient's biological response to orthodontic treatment and influence susceptibility to conditions such as external apical root resorption (EARR) [3]. The importance of considering these factors is underscored by their potential connection to broader health issues, where ethnicity-specific responses are observed and described in several pathological conditions such as sickle cell disease in individuals of African descent [4]; thalassemia among Mediterranean, Middle Eastern, and Southeast Asian populations [5]; and diabetes, with higher incidence in Latino and Native American groups [6]. In today's increasingly globalized cities, where diverse ethnic backgrounds converge [7], it is crucial to address these genetic and demographic factors to provide culturally and biologically tailored health care.

Permanent loss of root mineral components through root resorption induced by orthodontic forces remains a clinically significant challenge. Despite extensive research, there is still no consensus regarding the risk factors associated with this condition [8], including tooth type, root morphology, and orthodontic therapy [3, 8]. However, susceptibility to EARR varies considerably among patients treated under similar protocols, suggesting that multiple factors, including ethnic background, might play a role in determining risk [3].

Morphological differences, including root length, crown-to-root ratio, and root shape, influence the distribution of mechanical forces during orthodontic treatment [9]. Teeth with shorter roots and lower crown/root ratios are thought to experience increased stress over a smaller surface area, which increases their susceptibility to root resorption [10]. These variations, shaped by both genetic factors and environmental conditions, are hypothesized to contribute to the ethnic differences in EARR susceptibility [11]. Although findings vary, studies have suggested that Caucasians and Hispanics exhibit more root resorption than other ethnic groups such as Asians [12]. However, owing to the inconsistencies in these results, more homogeneous studies focusing on specific populations and employing standardized treatment protocols are necessary to draw more reliable conclusions.

Given the varying susceptibility to EARR among different ethnic groups, it is crucial to ascertain whether ethnicity may act as a potential risk factor for this condition. Understanding ethnic variations in root resorption can lead to more accurate risk assessment and personalized treatment plans. Ethnicity-related differences in dental and bone morphology may contribute to different responses to orthodontic forces, and recognizing these differences is essential for optimizing patient care. This approach not only enhances the precision of orthodontic care, but also promotes equity in treatment outcomes across diverse populations, ultimately enhancing patient outcomes and advancing the field of orthodontics.

Therefore, the primary aim of the present study was to evaluate whether ethnic differences might influence susceptibility to external apical root resorption (EARR) secondary to orthodontic forces. Second, we aimed to evaluate whether differences were found in root morphology in an ethnically defined pattern.

MATERIAL AND METHODS

Study design, methodological guidelines and ethic statement

The present study fulfilled the STROBE guidelines and was designed as a cross-sectional study with a nested case-control analysis and was conducted on 200 out of 800 patients treated at the Orthodontic Department of the Complutense University in Madrid. The eligibility criteria for study participation are outlined in *Supporting Information File 1*. Approval was obtained from the Institutional Ethical Review Board of Clinical Hospital San Carlos, Madrid (IRB reference number: 17/038-E). Prior consent was obtained from each participant, allowing the use of their clinical, diagnostic, and radiographic data for health-related research as part of this study. This study was conducted in accordance with the ethical principles outlined in the Helsinki Declaration [13] governing medical research involving human subjects.

Study cohorts and variables analyzed

The sample was divided into three ethnic-demographic groups, based on recommendations from previous studies [14]: South European, Latin American, and East Europeans. This demographic classification was based on the diagnosis and self-reports from each patient, taking into account the first- and second-degree family history [14]. Six diagnostic variables and 11 treatment-related variables[3, 15] that have been previously associated with EARR were analyzed from patient records to adjust for confounding factors. (*Supporting Information File 1).*

Tooth measurement and EARR diagnosis

EARR was diagnosed using panoramic radiographs taken at the beginning and end of orthodontic treatment. Among the four upper incisors that are the most studied and susceptible to root resorption [3], the upper right central and lateral incisors (1.2 and 1.1) were measured in all patients. The most commonly method used in literature for diagnosing EARR in 2D was employed using Adobe Photoshop software: the Linge and Linge method, modified by Brezniak [16]. Also the Malgrem visual method was used to contrast the measurement results [17]. Additionally, the crown/root ratio of both incisors was calculated to assess the dental proportions [11].

Seven radiographic variables were recorded for each incisor. The Malgrem method was used to differentiate the sample into patients with a score of 0 (no EARR), patients with mild or moderate EARR (Malgrem 1,2), and patients with severe and extreme EARR (Malgrem 3,4).

Statistical analysis

To ensure the reliability of the radiographic measurement method, inter-observer variability was minimized by performing all measurements by the same experienced operator (P.I.). The method error for radiographic measurements was assessed by conducting a repeatability analysis. Measurements were obtained from panoramic radiographs of 40 randomly selected subjects, with a second set of measurements performed 20 days later. A paired Student's t-test was used to compare the two sets of measurements, with no significant difference between them considered evidence of consistency and reliability in the measurement method.

For the descriptive analysis, t-tests and chi-square tests were performed. Group comparisons were performed by using a backward stepwise binary logistic regression model. ANOVA was employed to analyze the qualitative variables.

RESULTS

Sample Characteristics and description

The study comprised 200 out of 800 initially screened patients, including 145 of South European origin, 39 of Latin American origin, and 16 of East European origin. The mean age of the sample was 13.75 years (SD ±5.10y). The average treatment duration was 28.12 months (SD±11.45m), with statistically significant differences between the Latin American and European groups. All groups showed sufficient homogeneity in age, sex, skeletal and molar classifications, and in the ABO Discrepancy Index prior to treatment initiation (*Table 1)*.

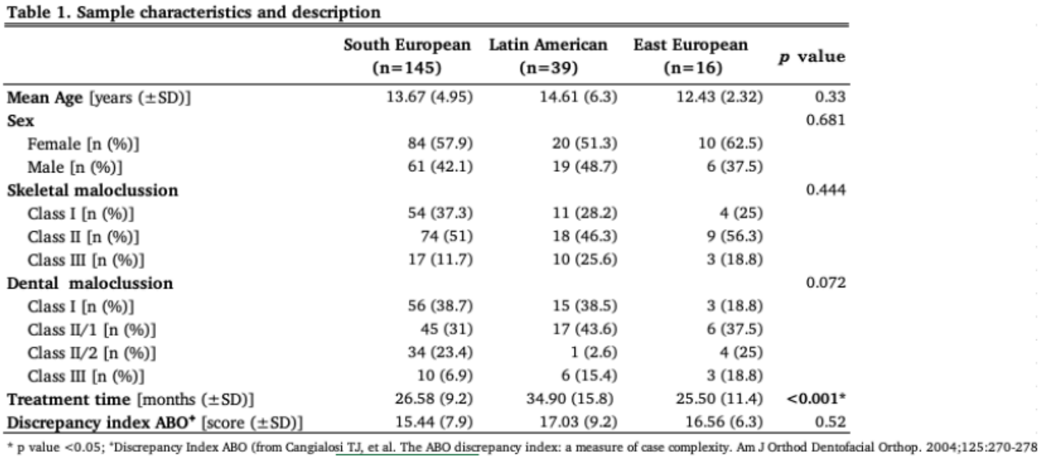


Table 1. Sample characteristics and description

	South European (n=145)	Latin American (n=39)	East European (n=16)	*p* value
Mean Age [years (±SD)]	13.67 (4.95)	14.61 (6.3)	12.43 (2.32)	0.33
Sex				0.681
Female [n (%)]	84 (57.9)	20 (51.3)	10 (62.5)	
Male [n (%)]	61 (42.1)	19 (48.7)	6 (37.5)	
Skeletal maloclussion				0.444
Class I [n (%)]	54 (37.3)	11 (28.2)	4 (25)	
Class II [n (%)]	74 (51)	18 (46.3)	9 (56.3)	
Class III [n (%)]	17 (11.7)	10 (25.6)	3 (18.8)	
Dental maloclussion				0.072
Class I [n (%)]	56 (38.7)	15 (38.5)	3 (18.8)	
Class II/1 [n (%)]	45 (31)	17 (43.6)	6 (37.5)	
Class II/2 [n (%)]	34 (23.4)	1 (2.6)	4 (25)	
Class III [n (%)]	10 (6.9)	6 (15.4)	3 (18.8)	
Treatment time [months (±SD)]	26.58 (9.2)	34.90 (15.8)	25.50 (11.4)	**<0.001***
Discrepancy index ABO[†] [score (±SD)]	15.44 (7.9)	17.03 (9.2)	16.56 (6.3)	0.52

* p value <0.05; [†]Discrepancy Index ABO (from Cangialosi TJ, et al. The ABO discrepancy index: a measure of case complexity. Am J Orthod Dentofacial Orthop. 2004;125:270-278

Ethnic differences in EARR, crown/root ratio and apex morphology

Several key variables have emerged as relevant for differentiating between ethnic groups. Notably, lateral incisor apex morphology with a pointed shape (p = 0.010, OR = 2.848, 95% CI 1.279-6.341) demonstrated differences (p<0,05) between Latin American and South European patients, indicating a greater prevalence of this feature in the first group. Additionally, South Europeans exhibited a higher rate of mild/moderate EARR severity (Malgrem score 1 and 2) than Latin American patients (p = 0,041 OR = 0.0385, 95% CI 0.154-0.961). Furthermore, South Europeans showed a greater crown/root ratio (1.46 ± 0,20) than East Europeans (1.33 ± 0,16) for the lateral upper incisor (12) (p = 0.007 OR = 0.016, 95% CI 0.001-0.326).

No significant differences (p<0,05) were found between the ethnic groups in the upper central incisor (1.1). However, the upper lateral incisor (1.2) showed clear distinctions in its apical morphology, severity of external apical root resorption, and crown/root ratio. Additionally, no notable differences were observed in the more aggressive forms of EARR severity among different ethnic groups (*Table 2)*.

Table 2. EARR, Crown/root ratio and appex morhology according ethnicity

		South European (n=145)	Latin American (n=39)	East European (n=16)	OR	95% CI		*p* value
						Min.	Max.	
11	**Malgrem score**							
	no EARR [n (%)]	53 (36.6)	16 (41)	8 (50)	a: 535854917.621 b: 2.583 c: 0.437	a: 0 b: 0.587 c: 0.139	a: - b: 11.355 c: 1.373	a: 0.998 b: 0.209 c: 0.156
	Mild/moderate EARR (1,2) [n (%)]	62 (42.8)	15 (38.5)	5 (31.3)	a: 569931063.512 b: 28.791 c: 0.783	a: 0 b: 0 c: 0.074	a: - b: - c: 8.239	a: 0.998 b: 1 c: 0.838
	Severe/extreme EARR (3,4) [n (%)]	30 (20.7)	8 (20.5)	3 (18.8)	a: - b: - c: -	a: - b: - c: -	a: - b: - c: -	a: 0.981 b: 0.882 c: 0.855
	Mean EARR							
	EARR < 4mm [n (%)]	131 (90.3)	31 (79.5)	14 (87.5)	a: 0 b: 0.208 c: 1.504	a: 0 b: 0.014 c: 0.186	a: - b: 3.166 c: 12.156	a: 0.998 b: 0.259 c: 0.702
	EARR > 4mm [n (%)]	14 (9.7)	8 (20.5)	2 (12.5)	a: 0 b: 0.208 c: 1.504	a: 0 b: 0.014 c: 0.186	a: - b: 3.166 c: 12.156	a: 0.998 b: 0.259 c: 0.702
	Crown/root ratio (±SD)	1.34 (0.18)	1.30 (0.20)	1.32 (0.19)	a: 0.852 b: 0.127 c: 7.225	a: 0.067 b: 0.002 c: 0.134	a: 10.814 b: 9.584 c: 389.519	a: 0.902 b: 0.350 c: 0.331
	Appex morphology							
	Curved [n (%)]	16 (11)	0 (0)	0 (0)	a: 0 b: - c: 2.195	a: 0 b: - c: 0.377	a: - b: - c: 12.795	a: 0.998 b: - c: 0.382
	Rounded [n (%)]	75 (51.7)	18 (46.2)	5 (31.3)	a: 0.931 b: 0 c: 2.764	a: 0.364 b:0 c: 0.875	a: 2.382 b: - c: 8.726	a: 0.881 b: 0.999 c: 0.083
	Pointed [n (%)]	50 (34.5)	18 (46.2)	9 (56.3)	a: 188575845.264 b: 0 c: 39339127.652	a: 0 b: 0 c: 0	a: - b: - c: -	a: 0,999 b: 0.999 c: 1
	Square [n (%)]	4 (2.8)	3 (7.7)	2 (12.5)	a: 4.470 b: 0 c: 731347984.824	a: 0.713 b: 0 c:0	a: 28.015 b: - c: -	a: 0.11 b: 0.999 c: 0.999
12	**Malgrem score**							
	no EARR [n (%)]	32 (22.1)	12 (30.8)	5 (31.3)	a: 1.812 b: 0.626 c: 0.936	a: 0.335 b: 0.027 c: 0.099	a: 9.800 b: 14.314 c: 8.862	a: 0.490 b: 0.769 c: 0.954
	Mild/moderate EARR (1,2) [n (%)]	72 (49.7)	14 (35.9)	8 (50)	a: 0.385 b: 2.869 c: 1.368	a: 0.154 b: 0.764 c: 0.368	a: 0.961 b: 10.775 c: 5.088	**A: 0.041*** b: 0.119 c: 0.640
	Severe/extreme EARR (3,4) [n (%)]	41 (28.3)	13 (33.3)	3 (18.8)	a: - b: - c: -	a: - b: - c: -	a: - b: - c: -	a: 0.538 b: 0.279 c: 0.417
	Mean EARR							
	EARR < 4mm [n (%)]	126 (86.9)	31 (79.5)	15 (93.8)	a: 0.756 b: 1.603 c: 0.389	a: 0.223 b: 0.112 c: 0.044	a: 2.564 b: 22.911 c: 3.467	a: 0.654 b: 0.728 c: 0.398
	EARR > 4mm [n (%)]	19 (13.1)	8 (20.5)	1 (6.3)	a: 0.756 b: 1.603 c: 0.389	a: 0.223 b: 0.112 c: 0.044	a: 2.564 b: 22.911 c: 3.467	a: 0.654 b: 0.728 c: 0.398
	Crown/root ratio (±SD)	1.46 (0.20)	1.42 (0.19)	1.33 (0.16)	a: 2.714 b: 13.040 c: 0.016	a. 0.360 b: 0.138 c: 0.001	a: 20.472 b: 1227.831 c: 0.326	a: 0.333 b: 0.268 **C: 0.007***
	Appex morphology							
	Curved [n (%)]	64 (44.1)	11 (38.2)	5 (31.3)	a: 1.483 b: - c: 0	a: 0.497 b: - c: 0	a: 4.422 b: - c: -	a: 0.480 b: 0.051 c: 0.999
	Rounded [n (%)]	39 (33.8)	10 (25.6)	5 (31.3)	a: 52664565.524 b: 4.172 c: 0.710	a: 0 b: 0.701 c: 0.178	a: - b: 24.845 c: 2.821	a: 0.999 b: 0.117 c: 0.626
	Pointed [n (%)]	32 (22.1)	17 (43.6)	6 (37.5)	a: 2.848 b: 1.112 c: 0.619	a: 1.279 b: 0.128 c: 0.144	a: 6.341 b: 9.666 c: 2.652	**A: 0.010*** b: 0.923 c: 0.518
	Square [n (%)]	0 (0)	1 (2.6)	0 (0)	a: 12490574210.01 b: 0 c: -	a: 0 b: 0 c: -	a: - b: - c: -	a: 1 b: 1 c: -

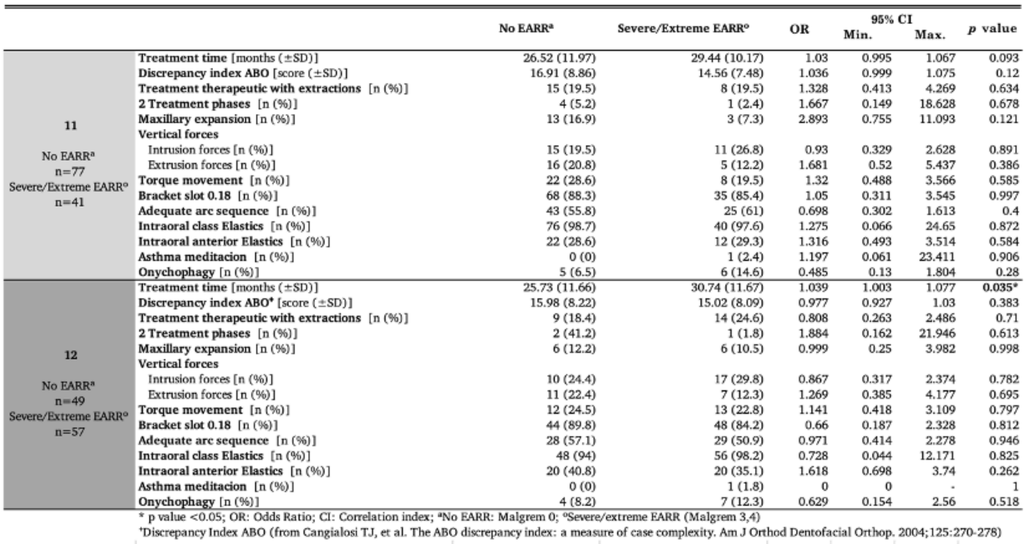
* p value <0.05; OR: Odds Ratio; CI: Correlation index; a: South Europe vs Latin American; b: Latin American vs East European; c: East vs South European;

Diagnostic and treatment related risk factors related to EARR

Analysis of covariables associated with external apical root resorption (EARR) in the central and lateral upper incisors revealed that treatment duration plays a key role in the development of severe/extreme EARR. In teeth 1.2, a longer treatment time was correlated with an increased risk of EARR (p = 0.035, OR = 1.039, 95% CI 1.003-1.077), suggesting that extended orthodontic treatment is a contributing factor to more severe cases of resorption (Grade 3,4). (*Table 3)*.

In contrast, variables such as the ABO Discrepancy Index, treatment with extractions, orthodontic treatment in two phases, maxillary expansion, application of vertical forces (intrusion or extrusion), torque movement, bracket slot size, adequate arc sequence, intraoral elastics, asthma medication, and onychophagy did not have a significant impact on the severity of EARR in the present analyzed sample (p<0,05) (*Table 3)*.

Table 3. Confounding factors related to EARR in central and lateral upper incisors (11,12)



		No EARR[a]	Severe/Extreme EARR[o]	OR	95% CI Min.	95% CI Max.	*p* value
11 No EARR[a] n=77 Severe/Extreme EARR[o] n=41	**Treatment time** [months (±SD)]	26.52 (11.97)	29.44 (10.17)	1.03	0.995	1.067	0.093
	Discrepancy index ABO [score (±SD)]	16.91 (8.86)	14.56 (7.48)	1.036	0.999	1.075	0.12
	Treatment therapeutic with extractions [n (%)]	15 (19.5)	8 (19.5)	1.328	0.413	4.269	0.634
	2 Treatment phases [n (%)]	4 (5.2)	1 (2.4)	1.667	0.149	18.628	0.678
	Maxillary expansion [n (%)]	13 (16.9)	3 (7.3)	2.893	0.755	11.093	0.121
	Vertical forces						
	Intrusion forces [n (%)]	15 (19.5)	11 (26.8)	0.93	0.329	2.628	0.891
	Extrusion forces [n (%)]	16 (20.8)	5 (12.2)	1.681	0.52	5.437	0.386
	Torque movement [n (%)]	22 (28.6)	8 (19.5)	1.32	0.488	3.566	0.585
	Bracket slot 0.18 [n (%)]	68 (88.3)	35 (85.4)	1.05	0.311	3.545	0.997
	Adequate arc sequence [n (%)]	43 (55.8)	25 (61)	0.698	0.302	1.613	0.4
	Intraoral class Elastics [n (%)]	76 (98.7)	40 (97.6)	1.275	0.066	24.65	0.872
	Intraoral anterior Elastics [n (%)]	22 (28.6)	12 (29.3)	1.316	0.493	3.514	0.584
	Asthma meditacion [n (%)]	0 (0)	1 (2.4)	1.197	0.061	23.411	0.906
	Onychophagy [n (%)]	5 (6.5)	6 (14.6)	0.485	0.13	1.804	0.28
12 No EARR[a] n=49 Severe/Extreme EARR[o] n=57	**Treatment time** [months (±SD)]	25.73 (11.66)	30.74 (11.67)	1.039	1.003	1.077	**0.035***
	Discrepancy index ABO[†] [score (±SD)]	15.98 (8.22)	15.02 (8.09)	0.977	0.927	1.03	0.383
	Treatment therapeutic with extractions [n (%)]	9 (18.4)	14 (24.6)	0.808	0.263	2.486	0.71
	2 Treatment phases [n (%)]	2 (41.2)	1 (1.8)	1.884	0.162	21.946	0.613
	Maxillary expansion [n (%)]	6 (12.2)	6 (10.5)	0.999	0.25	3.982	0.998
	Vertical forces						
	Intrusion forces [n (%)]	10 (24.4)	17 (29.8)	0.867	0.317	2.374	0.782
	Extrusion forces [n (%)]	11 (22.4)	7 (12.3)	1.269	0.385	4.177	0.695
	Torque movement [n (%)]	12 (24.5)	13 (22.8)	1.141	0.418	3.109	0.797
	Bracket slot 0.18 [n (%)]	44 (89.8)	48 (84.2)	0.66	0.187	2.328	0.812
	Adequate arc sequence [n (%)]	28 (57.1)	29 (50.9)	0.971	0.414	2.278	0.946
	Intraoral class Elastics [n (%)]	48 (94)	56 (98.2)	0.728	0.044	12.171	0.825
	Intraoral anterior Elastics [n (%)]	20 (40.8)	20 (35.1)	1.618	0.698	3.74	0.262
	Asthma meditacion [n (%)]	0 (0)	1 (1.8)	0	0	-	1
	Onychophagy [n (%)]	4 (8.2)	7 (12.3)	0.629	0.154	2.56	0.518

* p value <0.05; OR: Odds Ratio; CI: Correlation index; [a]No EARR: Malgrem 0; [o]Severe/extreme EARR (Malgrem 3,4)

[†]Discrepancy Index ABO (from Cangialosi TJ, et al. The ABO discrepancy index: a measure of case complexity. Am J Orthod Dentofacial Orthop. 2004;125:270-278)

DISCUSSION

Ethnic differences significantly influence an individual's susceptibility to EARR (External Apical Root Resorption) [18], highlighting the importance of investigating ethnicity in medical research. As personalized treatment strategies become more prevalent, ethnicity has become increasingly vital. Expanding knowledge of disease risks and prevalence among diverse populations is crucial for mitigating the broader impact of the disease. In today's globalized world, understanding ethnic and genetic variations is more important than ever [19].

In this study, the population was categorized based on biogeographical criteria at the continental level [20]. This approach aligns with the use of ancestry-informative markers (AIMs), which is a widely accepted method for identifying ancestry. AIMs are often correlated with predefined regional or population groups or collections of populations that are geographically and linguistically similar [21-23].

Genetic susceptibility, which might influence the occurrence of certain pathologies or conditions, has been described to follow an ethnic-dependent trend in certain populations, as is the case with severe periodontal disease. Thus, it is critical to consider population-specific, likely risk gene variants [24]. Genetic factors have been reported to account for up to 80% of the variance in bone mass, with chromosomal regions associated with bone mineral density [25, 26], which is another factor influencing the development of EARR [27]. Ethnic differences in bone mineral density have been linked to genetic variations affecting vitamin D metabolism and osteoporosis risk, particularly in individuals of Latin American ancestry [26, 28, 29]. These differences may also extend to the expression of inflammatory markers such as interleukin-6 (logIL-6) and C-reactive protein (logCRP), which are inflammatory cytokines known to negatively impact bone health. However, a study investigating whether central adiposity or inflammatory status contributes to ethnic differences in bone mineral density found no significant differences [29].

Studies have reported differences in inflammatory cytokine levels across racial and ethnic groups in the United States [30]. Research shows that Black and Latinx populations have the highest mean CRP levels, often attributed to genetic differences, and inflammatory mechanisms in systemic diseases, such as type 2 diabetes, are influenced by complex factors tied to race and ethnicity [30]. However, environmental and social factors affecting minority groups, such as pollution, limited access to education, healthy food, and safe living conditions, must also be considered [30]. It has been hypothesized that inflammation could serve as a common link between socioeconomic status, cardiovascular disease, and other aging-related conditions, as individuals of lower socioeconomic status bear a greater inflammatory burden than those of higher socioeconomic status, driven by behavioral, psychosocial, and metabolic factors [31].

The method and accuracy of diagnosing EARR using panoramic radiographs are widely accepted because of their ability to capture all teeth in a single image despite some limitations related to distortion and magnification[3]. This approach is a practical tool for orthodontic assessment. Focusing on the upper incisor with the most pronounced EARR and clearest definition is justified, as these teeth are particularly susceptible to resorption and are typically well visualized on panoramic radiographs. The technique used, based on the Linge and Linge method and modified by Brezniak [16], provides an efficient balance between accuracy and practicality in clinical settings. Defining significant EARR as resorption exceeding 2 mm, in line with established thresholds, allows for standardization across studies. Although two-dimensional imaging does not capture certain aspects, such as buccal or lingual resorption, this method still offers reliable assessments. Future improvements could include the incorporation of more precise imaging techniques and evaluation of multiple teeth to enhance the thoroughness of EARR analysis across diverse populations.

Root morphology has been identified as a significant risk factor for the development of EARR [27] and ethnicity has been shown to influence tooth shape and anatomy [32]. In our study, no ethnic differences were found in the upper central incisor; however, differences were observed in the upper lateral incisor, consistent with previous findings that suggest it may be more predisposed to morphological variations [33].

Furthermore, our results showed that Latin American patients exhibited a higher prevalence of pointed apex morphology ($p = 0.010$) and a lower rate of mild/moderate EARR severity than South European patients. This observation raises the intriguing possibility that pointed apex morphology might serve as a protective factor against EARR, underscoring the need for further research to explore this potential relationship. Such morphological traits could play a critical role in EARR development, as suggested by previous studies on ethnic variations in dental anatomy [11, 34-36]. Variations in tooth shape have been documented across different populations, with "white" populations tending to exhibit smaller tooth dimensions [37]. In addition, a morphometric analysis of tooth morphology in "Hispanic" patients revealed notable 3D shape differences among those with Angle Class I, Class II, and Class III malocclusions compared to other populations [37]. These findings support the notion that the morphological differences associated with ethnicity may influence susceptibility to EARR, highlighting the importance of considering these traits in orthodontic risk assessments.

Root canal anatomy has also been found to vary among populations [38], and a decrease in the root-to-crown ratio is thought to increase the load on the root, resulting in significant stress [39]. In the present study, the crown/root ratio was higher in South European patients ($p = 0.007$); however, this group was also the most susceptible to mild/moderate EARR severity. In contrast, some studies reported lower ratios in "Hispanic" patients than in African Americans and Caucasians [40]. However, more recent studies have shown much higher root-to-crown ratios in Puerto Rican patients [40]. This highlights the need for further studies to clarify whether the crown/ratio contributes to the differential risk of EARR.

Lastly, it is noteworthy that no significant differences were found in the most aggressive forms of EARR severity across different ethnic groups. This lack of variability suggests that, while mild to moderate EARR may be influenced by ethnic and morphological factors, the progression to more severe forms might be governed by factors unrelated to ethnicity, warranting further investigation.

CONCLUSIONS

This study highlights the influence of ethnicity on susceptibility to external apical root resorption (EARR), particularly apical morphology, crown/root ratio, and severity of EARR in the upper lateral incisor. Latin American patients displayed a higher prevalence of pointed apical morphology in the upper lateral incisor and a lower incidence of mild/moderate EARR than European patients, emphasizing the importance of incorporating ethnic factors in orthodontic risk assessments. Additionally, South European patients showed a significantly greater crown/root ratio in the upper lateral incisor than East European patients.

Furthermore, treatment duration emerged as a critical factor associated with severe/extreme EARR, with longer treatment times correlating with heightened risk.

Notably, however, no significant ethnic differences were observed in the most aggressive forms of EARR, suggesting that while ethnic and morphological factors may influence mild-to-moderate resorption, the progression to more severe resorption may be driven by other factors independent of ethnicity.

These findings underscore the importance of personalized treatment plans that consider ethnic variations in dental morphology and susceptibility to root resorption, ultimately aiming to improve patient outcomes and promote equity in orthodontic care.

Supplementary Information: *Supplementary File I*

Author contributions: All authors made substantial contribution to the conception and design of the manuscript and research. P.I.D. and A.D.S., wrote the main manuscript text, P.I.D and R.S.C.. made de data screening and collecting. J.C.P.F and A.I.L directed the research. All authors drafted the work and revised it critically for important intellectual content. All authors agree to be accountable for all aspects of the study design and its content. All authors approved the final submitted version.

Complaince with ethical standards:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: Approval was obtained from the Institutional Ethical Review Board of Clinical Hospital San Carlos, Madrid (IRB reference number: 17/038-E). This study was conducted in accordance with the ethical principles outlined in the Helsinki Declaration [13] governing medical research involving human subjects.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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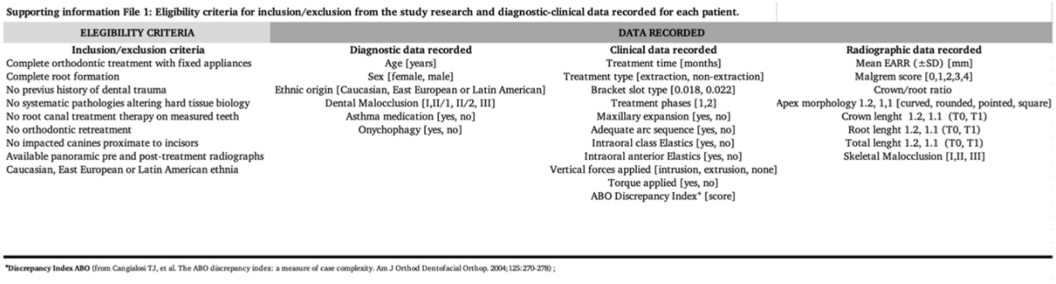
Suppoting information File 1*: Eligibility criteria for inclusion/exclusion. From the study research and diagnostic-clinical data recorded for each patient.*



Supporting information File 1: Eligibility criteria for inclusion/exclusion from the study research and diagnostic-clinical data recorded for each patient.

ELEGIBILITY CRITERIA	DATA RECORDED		
Inclusion/exclusion criteria	**Diagnostic data recorded**	**Clinical data recorded**	**Radiographic data recorded**
Complete orthodontic treatment with fixed appliances	Age [years]	Treatment time [months]	Mean EARR (±SD) [mm]
Complete root formation	Sex [female, male]	Treatment type [extraction, non-extraction]	Malgrem score [0,1,2,3,4]
No previus history of dental trauma	Ethnic origin [Caucasian, East European or Latin American]	Bracket slot type [0.018, 0.022]	Crown/root ratio
No systematic pathologies altering hard tissue biology	Dental Malocclusion [I,II/1, II/2, III]	Treatment phases [1,2]	Apex morphology 1.2, 1,1 [curved, rounded, pointed, square]
No root canal treatment therapy on measured teeth	Asthma medication [yes, no]	Maxillary expansion [yes, no]	Crown lenght 1.2, 1.1 (T0, T1)
No orthodontic retreatment	Onychophagy [yes, no]	Adequate arc sequence [yes, no]	Root lenght 1.2, 1.1 (T0, T1)
No impacted canines proximate to incisors		Intraoral class Elastics [yes, no]	Total lenght 1.2, 1.1 (T0, T1)
Available panoramic pre and post-treatment radiographs		Intraoral anterior Elastics [yes, no]	Skeletal Malocclusion [I,II, III]
Caucasian, East European or Latin American ethnia		Vertical forces applied [intrusion, extrusion, none]	
		Torque applied [yes, no]	
		ABO Discrepancy Index* [score]	

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*Discrepancy Index ABO** (from Cangialosi TJ, et al. The ABO discrepancy index: a measure of case complexity. Am J Orthod Dentofacial Orthop. 2004;125:270-278) ;

8. CAPÍTULO 3

Predisposición genética a la reabsorción radicular apical externa: GWAS.

Paper #3. Iber-Diaz P, Senen-Carramolino R, Iglesias-Linares A, Fernandez-Navarro P, Flores-Mir C, Yanez-Vico RM. (2020). GWAS of Post-Orthodontic Aggressive External Apical Root Resorption Identified Multiple Putative Loci at X-Y Chromosomes. *Journal of Personalized Medicine*; 10,4. doi: 10.3390/jpm10040169

Paper #4. Iber-Diaz P, Senen-Carramolino R, Fernandez-Navarro P, Palma-Fernandez JC, Iglesias-Linares A (2024). GWAS of post-orthodontic aggressive external apical root resorption. [In preparation]. *Nature genetics.*

RESUMEN:

El tercer capítulo se centra en el estudio de la reabsorción radicular apical externa agresiva (aEARR) desarrollada tras el tratamiento de ortodoncia, y en la identificación de las bases genéticas que podrían estar implicadas en su aparición. Está compuesto por dos publicaciones científicas que dan respuesta a la hipótesis planteada: variantes genómicas específicas ubicadas a lo largo del genoma, incluidos los cromosomas sexuales cromosomas X e Y, están asociadas con un riesgo variable a desarrollar aEARR.

Para abordar esta cuestión, se llevaron a cabo dos análisis de asociación genética: en 480 pacientes en el primer GWAS y en 612 pacientes en el segundo GWAS, incluyendo un modelo multinivel con diversidad étnica, sobre múltiples loci y genes potenciales localizados en los cromosomas 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 15,18, 19, X e Y, implicados potencialmente en el desarrollo de aEARR secundaria a fuerzas ortodóncicas. Esto fue complementado con un análisis clínico detallado, además del diagnóstico de aEARR mediante la cuantificación radiográfica de las lesiones de reabsorción antes y después del tratamiento ortodóncico.

Los hallazgos obtenidos no solo refuerzan la hipótesis de que existe una base genética para esta patología, sino que se identificaron múltiples variantes genéticas implicadas en un fenotipo de aEARR, además, se observó una asociación vinculada al género.

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GWAS of Post-Orthodontic Aggressive External Apical Root Resorption Identified Multiple Putative Loci at X-Y Chromosomes

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Abstract: Personalized dental medicine requires from precise and customized genomic diagnostic. To conduct an association analysis over multiple putative loci and genes located at chromosomes 2, 4, 8, 12, 18, X, and Y, potentially implicated in an extreme type of external apical root resorption secondary to orthodontic forces (aEARR). A genome-wide association study of aEARR was conducted with 480 patients [ratio~1:3 case/control]. Genomic DNA was extracted and analyzed using the high-throughput Axiom platform with the GeneTitan® MC Instrument. Up to 14,377 single nucleotide polymorphisms (SNPs) were selected at candidate regions and clinical/diagnostic data were recorded. A descriptive analysis of the data along with a backward conditional binary logistic regression was used to calculate odds ratios, with 95% confidence intervals [$p < 0.05$]. To select the best SNP candidates, a logistic regression model was fitted assuming a log-additive genetic model using R software [$p < 0.0001$]. In this sample the top lead genetic variants associated with aEARR were two novel putative genes located in the X chromosome, specifically, STAG 2 gene, rs151184635 and RP1-30E17.2 gene, rs55839915. These variants were found to be associated with an increased risk of aEARR, particularly restricted to men [OR: 6.09; 95%CI: 2.6–14.23 and OR: 6.86; 95%CI: 2.65–17.81, respectively]. Marginal associations were found at previously studied variants such as *SSP1*: rs11730582 [OR: 0.54; 95%CI: 0.34–0.86; $p = 0.008$], *P2RX7*: rs1718119 [OR: 0.6; 95%CI: 0.36–1.01; $p = 0.047$], and *TNFRSF11A*: rs8086340 [OR: 0.6; 95%CI: 0.38–0.95; $p = 0.024$]), found solely in females. Multiple putative genetic variants located at chromosomes X and Y are potentially implicated in an extreme phenotype of aEARR. A gender-linked association was noted.

Keywords: orthodontics; dentistry; dental trauma; resorption; fixed appliances

1. Introduction

External apical root resorption (EARR) resulting from orthodontic forces represents one of the most undesirable iatrogenic effects secondary to mechanical strain during orthodontic movement, provoking an irreversible loss of root structure and tooth attachment in the apical third of the tooth root [1,2]. EARR is mostly manifested in its mild to moderate forms [3–5]; however, the most aggressive phenotype, with a frequency <1–5% and >5 mm apical loss, might critically compromise tooth viability [6,7].

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EARR of any degree represents a complex pathological trait with multilevel etiological-risk factors that have not been completely elucidated to date [8]. Several diagnostic and clinical factors have been associated with EARR [9]. Several factors, such as treatment time, apical displacement, and gender-specific risk have been found to be associated with EARR, but these findings systematically show some degree of inconsistency and controversy in literature [10–12]. In fact, EARR occurrence and severity remains unpredictable and is not fully explained by clinical evidence alone. In this context, a genetic component and its contribution to this pathological feature, has been a critical issue that has recently garnered considerable attention [13,14]. Particularly, risk loci in somatic chromosomes have been targeted extensively, whereas very few studies are available regarding regions potentially associated with EARR at sexual chromosomes [15]. To date, a limited number of candidate gene association studies [15–24] have provided evidence suggesting that some genetic variants might exert a positive or negative influence over EARR of a mild to moderate degree at the level of the *IL1* gene cluster [15,20–24], *TNFRSF11B* [25], *P2RX7* [17,18], *SSP1* [19,23], or *TNFRSF11A* [15,26] at an autosomic level but also at *IRAK1* gene [27] on sexual chromosome X. Despite the above mentioned studies, there is no available scientific evidence regarding how genetic factors might be specifically associated with the most severe phenotype of EARR, i.e., aggressive EARR (aEARR). Moreover, whether aEARR is positively/negatively associated with previous genetic variants and the studied clinical/diagnostic factors remains to be elucidated.

Therefore, the present study aimed to perform the first genome-wide association study conducting an association analysis over multiple putative loci and genes located at somatic chromosomes 2, 4, 8, 12, 18, and at sexual chromosomes X and Y, potentially implicated in aEARR.

2. Materials and Methods

2.1. Study Design

We performed a genome-wide association study of aEARR, a derived extreme and well-delimited root resorption phenotype, in $UCM_{3D}{}^{g}$ consortium participants (Figure 1). The $UCM_{3D}{}^{g}$ consortium database is based on a general-population cohort of roughly 0.01 million patients aged 9–67 years old, recruited across Spain between 2005 and 2019.

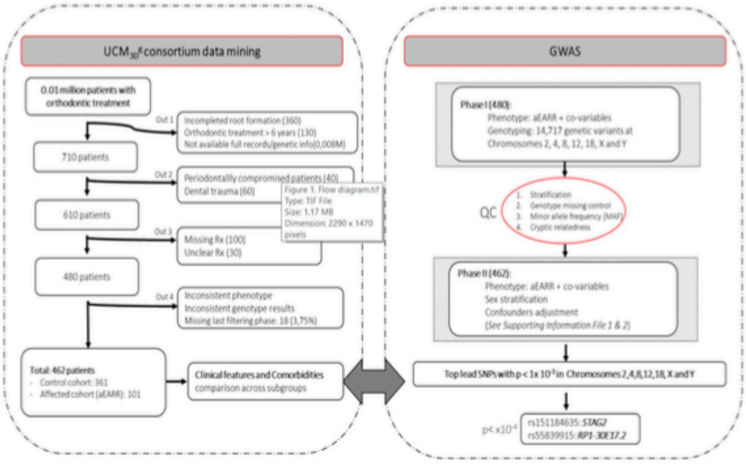

Figure 1. Flow diagram of the filtering process and genome-wide association study.

2.2. Sample Size, Study Cohorts, and Ethics Statement

2.2.1. Sample Size Calculation

Sample size estimation was based on the minimum sample size required for a genetic association study based on: (a) the prevalence of the disease: 0.01; (b) ratio of cases to controls (~1:3); (c) alpha error 1×10^{-4}/beta error: <0.20; (d) base relative risk: 2.9; and (f) frequency of risk allele: 0.25. Sample size was calculated using the freely available Genetic Power Calculation software [28]. It was determined that 450 patients would be needed to establish an association between the presence of a single nucleotide polymorphism (SNP) of interest under these conditions and the appearance of an advanced state of EARR, 100 cases for the aggressively affected cohort and 350 controls, with 3% overestimation to include expected dropouts were required.

2.2.2. Cohort Distribution and Ethics Statement

The study population comprised 480 patients [*ratio ~1:3; case:control*] with available radiographic, diagnostic, and clinical records from the $\text{UCM}_{3D}{}^{g}$ consortium database, who were eligible for participation in the present study and met the inclusion criteria detailed in *Supporting Information File 1*. Up to 4.8% of the eligible $\text{UCM}_{3D}{}^{g}$ consortium patients with available diagnostic, clinical, and radiographic records were enrolled with the complete available genetic data used in the present study. We were granted approval from the Institutional Ethical Review Board of the Clinical Hospital San Carlos, Madrid (IRB) [ref#:17/038-E] and permission was previously obtained from each individual to participate in the present study, allowing their clinical, diagnostic, radiographic, and genomic data to be used for health-related research as part of this study. This study was carried out in accordance with the ethical principles governing medical research and human subjects, as laid down in the Helsinki Declaration (Helsinki Declaration 2013 version. Available online: https://jamanetwork.com/journals/jama/fullarticle/1760318 (accessed on 3 March 2017),). Details of the consenting process are described elsewhere [29].

2.3. *Knowledge Discovery in $UCM_{3D}{}^{g}$ and $SALUD^{R}$ Databases: Data Mining*

Quality Control (QC), Data Filtering, and Extraction

A pre-piloted protocol was followed for data filtering and extraction from the $\text{UCM}_{3D}{}^{g}$ and SALUD^{R} databases. As detailed in *Supporting Information File 2*, the diagnostic variables, clinical setting variables, and radiological values were collected and subjected to QC (accession registered URI+i *ref#:5-201119*). Manifest-codifying data errors in the database were removed by selecting and identifying implausible values in each category of diagnostic, clinical, or radiological variables (e.g., Discrepancy index (DI) = 400) or others that were not adequately recorded in terms of the type of unit or quantitative/categorical format (e.g., age = a). Extreme values of duration of mechanical loading (treatment time > 72 months) justified elimination as they were more likely to be data errors or non-physiological extremes rather than feasible variable inputs. The quality-checked data were processed in the next steps (Figure 1).

2.4. *Phenotyping and Radiographic Measurements*

All subjects selected for final inclusion in the study were assigned to the affected or control cohorts of patients according to radiological screening, using radiographic measurements performed in duplicate and in a double-blinded (P.I. and R.S) manner on orthopantomographic and teleradiographic projections that were already available and used at the clinic for routine diagnosis and treatment. Subjects were classified and divided into these two groups, based on the presence or absence of the phenotype of aggressive post-orthodontic EARR of more than 5 mm in blinded radiographic measurements. The affected cohort included patients with severe EARR $\geq$ 5 mm and the control group was composed of patients with EARR < 5 mm [7].

The following methods have been detailed previously; an aEARR phenotype was assessed after the roots were measured from before and after treatment on panoramic radiographs focusing on the maxillary central and lateral incisors [4,5,15]. All pre- and post-treatment images were calibrated beforehand and a correction factor for magnification was applied in all cases. Measurements were performed on digital radiographs using diagnostic software (Adobe Photoshop CS8, Adobe Systems

Incorporated, San Jose, CA, USA) enabling the image filters to provide maximum precision when localizing the terminal points of the roots. Accordingly, the tooth with the highest EARR value was selected as the dependent variable of interest for that subject using the method described by Linge and Linge [30], modified by Brezniak et al., (2004) [31]. Pre- and post-treatment radiographs through the initial and final root (r1 and r2, respectively) and crown (c1 and c2, respectively) lengths were used to determine the changes in dental and root length. If the root became shorter during treatment, the EARR value resulted from r1–r2 [c1/c2]).

Apical displacement and variation in tooth inclination were quantified using the superimposition of radiographic measurements on a lateral radiograph, using a modified version of the method described by Baccetti et al., (1998) [32].

2.5. Genotyping

DNA Extraction, Genotyping

Genomic DNA was extracted from saliva according to the manufacturer's instructions (prepIT•L2P, DNA Genotek, Ottawa, ON, Canada). Total genomic DNA was checked for purity and integrity [OD_{260}/OD_{280}: 1.8–2.0; $OD_{260}/OD_{230} > 1.5$; 1% agarose gel integrity: 90% DNA size > 10Kb]. Quality control was then performed per sample using the *Agena Bioscience MassARRAY plattform iPLEX GOLD technology* to eliminate samples with poor quality.

DNA samples were genotyped using the high-throughput *Axiom* platform with the GeneTitan® MC Instrument *(Axiom Genome-Wide Human Assay technology, CeGen)*. This method has been extensively validated in literature [33,34].

Data were analyzed, using the Axiom Analysis Suite 4.0 software for genotype clustering and calling. We then implemented a quality control for the data, where we checked possible sample stratification, missing SNP genotype, SNP monomorphic status, and SNP minor allele frequency (*see Supporting Information File* 3) from 687,133 markers. Next, to achieve our objectives, we selected 14,377 SNPs located in the X and Y-chromosomes along with other candidate genes at chromosomes 2, 4, 8, 12, and 18 for the analysis (*see Supporting Information File* 4).

2.6. Statistics

2.6.1. Overall Statistical Analysis of Clinical and Radiological Variables

A descriptive analysis of the data for quantitative and categorical variables based on diagnostic or treatment factors was performed (mean, SD, ranges, frequencies, and distributions). Backward conditional binary logistic regression was used to assess the extent to which specific diagnostic and treatment parameters interfere within the observed aEARR process; odds ratios (OR) with a 95% confidence interval were also calculated. SPSS was used for data analysis (version 22.0; LEAD Technologies, Chicago, IL, USA), with statistical significance set at a value of $p < 0.05$ [35].

2.6.2. Genetic Association Tests

To select our best SNP candidates, we fitted a logistic regression model adjusted by the type of treatment, total length of treatment (load duration), and gender, and assumed a log-additive genetic model. These statistical analyses were performed using R software (R Core Team, Vienna, Austria. version 3.6.1) [35].

2.6.3. Reliability and Accuracy of the Measurement Method

To prevent inter-observer variation, the same experienced operator (R.S) carried out all the measurements defined previously. However, a second experienced examiner (P.I) replicated the measurements on a subset of 50 patients to calculate the inter-observer error. The *kappa* coefficient was analyzed to determine concordance between EARR-affected and non-affected group assignment, based on radiological screening, and the result was a value of one. The method error was also calculated for measurements acquired from radiographic records, comparing double measurements of 20 randomly chosen subjects at an interval of 20 days. A paired Student's t-test was used for calculations, with an absence of significance being regarded as indicative of concordance between repeated measurements; the intraclass correlation coefficient (ICC) for absolute agreement was also calculated for both intra- and inter-observer errors. Accuracy of measurement was obtained from the equation:

$$SE = \sqrt{}(\Sigma d2/2n)$$

where d is the difference between the double measurements and n is the number of paired double measurements [36].

3. Results

3.1. Phenotyping: Reliability of the Radiographic Measurement Methods and the Associated Errors

aEARR was phenotyped according to radiographic measurements with a threshold for root resorption value greater than 5 mm. Reliability of the measurements retrieved no statistically significant differences between replicated assessments ($p > 0.05$) and intraclass correlation coefficient (ICC: 0.930), and the concordance index resulted in optimal values ($k = 1.00$) for both intra- and inter-examiner measurements, respectively [37]. Method error for measurements obtained from panoramic radiographs following the described method was calculated to be below <0.04 mm.

3.2. Sample Characteristics, Description and Analysis of aEARR Risk Associated with Clinical Features

The flow chart diagram (Figure 1) describes the sampling filtering steps and the following research strategy. The present study sample included a cohort of patients with relatively homogenous diagnostic characteristics, who had undergone mechanical load during a full orthodontic treatment, as detailed in Table 1. The mean ages of patients treated in the affected and control cohorts were ~21 ± 12 and ~23 ± 12 years old, respectively, with a fair balance found for sex distribution. The American Board of Orthodontics (ABO) discrepancy index was found to be quite homogenous in both groups [~16 ± 9 and ~15 ± 8] with a mean treatment time of ~27 ± 9 and ~25 ± 8 months, respectively (Table 1). Results from the associative testing by regression analysis are detailed in Table 1. The results showed that recorded clinical factors do not confer an additional risk of aEARR when compared to the control cohort. Specifically, differences in treatment time [OR: 0.974; 95%CI: 0.944–1.005; $p = 0.095$] or treatment type, extraction vs. non-extraction [OR: 1.668; 95%CI: 0.839–3.316; $p = 0.145$], did not confer an additional risk for aEARR process when results from both the study cohorts in the regression analysis were compared.

3.3. Genotype Distributions and Analysis of aEARR Risk Associated with Genetic Variants at Multiple Putative Loci on Chromosomes 2, 4, 8, 12, 18, X, and Y.

In addition to diagnostic and treatment-related co-variables, patients included in the present study were genotyped for specific novel target genetic variants and other SNPs explored in previous studies with regard to a risk of any degree of EARR found along the genome in chromosomes 2, 4, 8, 12, 18, X, and Y (*see Supporting Information File 3*).

When the whole cohort sample was explored within the associated risk for aEARR, a gender-dependent effect was detected to influence the results. The top lead genetic variants associated with aEARR [p value $< 1 \times 10^{-4}$], after gender stratification, focused on two novel putative genes located in the X chromosome, specifically, *STAG 2* gene, stromal antigen 2 gene, rs151184635 (*prior* to and after adjustment for confounding factors) and *RP1-30E17.2*, clone-based (Vega) gene rs55839915 (after adjustment for confounding factors). These two target variants were found to be associated with an increased risk of aEARR; this effect was particularly restricted to men [OR: 6.09; 95% CI: 2.6–14.23 and OR: 6.86; 95% CI: 2.65–17.81, respectively] (Tables 2 and 3).

Table 1. Population demographics, diagnostic and clinical characteristics of the included patients.

D & Cl Parameters	aEARR¶ Cohort (n = 101)	Control Group (n = 361)	p Value **	OR	95% CI Lower	95% CI Upper
Mean age [years]	21.52 ± 11.65	22.83 ± 11.66	0.067	1024	0.998	1051
Sex [n (%)]			0.209	1413	0.824	2.42
female	51 (50.49%)	205 (56.78%)				
male	50 (49.50%)	156 (43.21%)				
Angle classification [n (%)]			0.161	-	-	-
Class I	49 (48.51%)	184 (51.80%)	0.628	1149	0.655	2018
Class II	39 (38.61%)	153 (42.38%)	1	0.49	0.209	1145
Class III	13 (12.87%)	24 (6.64%)				
Treatment [n (%)]			0.145	1668	0.839	3316
extraction	20 (19.80%)	59 (16.34%)				
non-extraction	81 (80.19%)	302 (83.65%)				
Treatment time (m)	27.0 ± 9.10	25.43±7.96	0.095	0.974	0.944	1005
ABO Discrepancy index	15.92 ± 8.91	15.10±8.30	0.876	0.997	0.966	1030
Apical displacement						
Vertical (mm)	−4.44 ± 5.91	−2.83 ± 7.75	0.07	1045	0.996	1095
Sagittal (mm)	−0.57 ± 5.39	−0.25 ± 4.63	0.758	1009	0.953	1068
Vertical (mm) [absolute]	5.32 ± 4.57	4.85 ± 6.88	0.932	0.998	0.957	1041
Sagittal (mm) [absolute]	4.04 ± 3.89	3.31 ± 3.12	0.182	0.951	0.883	1024

D&Cl: Diagnostic and clinical factors; aEARR: aggresive external apical root resorption secondary to mechanical strain; m: months; ABO: American Board of Orthodontics; DI: ABO Discrepancy index (*from Cangialosi TJ*, et al. *The ABO discrepancy index: a measure of case complexity. Am J Orthod Dentofacial Orthop. 2004;125:270-278)*; ¶ at least one maxillary incisor with EARR lesion > 5 mm; **: Conditional backward binary logistic regression analysis. Dependent variable = control vs affected patients.

Table 2. Lead genetic variants associated with aggresive external apical root resorption [*stratified by sex*] *.

Stratification Code	Lead SNP	OR	Lower	Upper	p-Value $^{£}$	FDR	Chromosome	Gene Name; Source and Description
Male	rs111826558	3.8	1.74	8.21	0.000959316	0.894103771	Chr. X	DMD; dystrophin [Source:HGNC Symbol;Acc:2928]
	rs705896	3.6	1.82	7.27	0.000147132	0.522979691	Chr. X	-
	rs4828068	0.6	0.39	0.82	0.000773819	0.894103771	Chr. X	NOX1; NADPH oxidase 1 [Source:HGNC Symbol;Acc:7889]
	rs5911806	1.8	1.29	2.52	0.00056894	0.894103771	Chr. X	STAG2; stromal antigen 2 [Source:HGNC Symbol;Acc:11355]
	rs151184635	5.9	2.55	13.72	0.000033 **	0.480531966	Chr. X	STAG2; stromal antigen 2 [Source:HGNC Symbol;Acc:11355]
	rs5975024	4.5	1.92	10.37	0.00059286	0.894103771	Chr. X	RP1-30E17.2; Clone-based (Vega) gene
	rs55839915	6	2.37	15.12	0.000146319	0.522979691	Chr. X	RP1-30E17.2; Clone-based (Vega) gene
	rs5976834	4.4	1.85	10.46	0.000972951	0.894103771	Chr. X	LOC107985698
	rs926809	3.8	1.92	7.58	0.00013632	0.522979691	Chr. X	-
	rs6636278	3.3	1.7	6.49	0.000407288	0.894103771	Chr. X	-
	rs5962226	5.2	2.22	12.02	0.000211995	1	Chr. X	-
	rs112512284	6.2	2.18	17.54	0.000760667	1	Chr. X	TMEM47; transmembrane protein 47 [Source:HGNC Symbol;Acc:18515]
Female	rs6521042	0.5	0.27	0.74	0.000767082	1	Chr. X	-
	rs7058787	0.4	0.26	0.74	0.000923093	1	Chr. X	-
	rs4827953	0.4	0.25	0.72	0.000889185	1	Chr. X	-
	rs2180271	0.4	0.21	0.65	0.000221128	1	Chr. X	TAF7L; TAF7-like RNA polymerase II, TATA box binding protein (TBP)-associated factor, 50 kDa [Source:HGNC Symbol;Acc:11548]
	rs6638162	2.5	1.52	4.16	0.00023748	1	Chr. X	-
	rs7049661	0.4	0.26	0.75	0.000964935	1	Chr. X	-
	rs28729587	2.8	1.48	5.4	0.000527269	1	Chr. X	SPRY3; sprouty homolog 3 (Drosophila) [Source:HGNC Symbol;Acc:11271]

* Not adjusted; $^{£}$Selected p value threshold $p < 1 \times 10^{-3}$; ** $p < 1 \times 10^{-4}$; OR: Odds Ratio; FDR: false discovery rate; Chr.: Chromosome.

Table 3. Lead genetic variants (adjusted) associated with aggresive external apical root resorption [*stratified by sex*].

Stratification Code	Lead SNP	OR	Lower	Upper	p-Value $^{£}$	FDR	Chromosome	Gene Name; Source and Description
Male	rs4892924	0.37	0.18	0.77	0.000687581	0.778251314	Chr. X	-
	rs62581812	0	0		0.000889678	0.778251314	Chr. X	FAM9B; family with sequence similarity 9, member B [Source:HGNC Symbol;Acc:18404]
	rs61463999	0	0		0.000250861	0.57740729	Chr. X	RP11-40F8.2; Clone-based (Vega) gene
	rs150255888	22.23	2.48	199.29	0.000806144	0.778251314	Chr. X	-
	rs705896	3.7	1.84	7.45	0.000141011	0.501260262	Chr. X	-
	rs5969333	0.44	0.25	0.77	0.000717117	0.778251314	Chr. X	-
	rs5956024	0	0		0.000217592	0.57740729	Chr. X	-
	rs4825856	0.3	0.11	0.83	0.000834288	0.778251314	Chr. X	GRIA3; glutamate receptor, ionotropic, AMPA 3 [Source:HGNC Symbol;Acc:4573]
	rs5911806	1.87	1.32	2.63	0.000324865	0.57740729	Chr. X	STAG2; stromal antigen 2 [Source:HGNC Symbol;Acc:11355]
	rs151184635	6.09	2.6	14.23	0,0000291**	0.41510095	Chr. X	STAG2; stromal antigen 2 [Source:HGNC Symbol;Acc:11355]
	rs5975024	4.93	2.09	11.67	0.000316819	0.57740729	Chr. X	RP1-30E17.2; Clone-based (Vega) gene
	rs55839915	6.86	2.65	17.81	0,0000641**	0.456213349	Chr. X	RP1-30E17.2; Clone-based (Vega) gene
	rs5976834	4.69	1.94	11.35	0.000695616	0.778251314	Chr. X	LOC107985698
Female	rs5962226	5.31	2.27	12.43	0.000179554	0.873550665	Chr. X	-
	rs112512284	6.47	2.25	18.6	0.000624115	0.873550665	Chr. X	TMEM47; transmembrane protein 47 [Source:HGNC Symbol;Acc:18515]
	rs4827953	0.42	0.24	0.72	0.000813414	0.873550665	Chr. X	-
	rs2180271	0.35	0.2	0.64	0.000178648	0.873550665	Chr. X	TAF7L; TAF7-like RNA polymerase II, TATA box binding protein (TBP)-associated factor, 50 kDa [Source:HGNC Symbol;Acc:11548]
	rs61736018	0	0		0.00090046	0.873550665	Chr. X	ARMCX4; armadillo repeat containing, X-linked 4 [Source:HGNC Symbol;Acc:28615]

$^{£}$ Selected p value threshold $p < 1 \times 10^{-3}$; **: $p < 1 \times 10^{-4}$; OR: Odds Ratio; FDR: false discovery rate; Chr.: Chromosome; Co-variables used for adjustment: Sex, Treatment time, Treatment type.

In addition to these two top genetic variants (as compiled in Tables 2 and 3), a total number of 27 novel genetic variants out of 14,717 [p value < 0.001] were identified with marginal association values, specifically in the sexual chromosomes; some of them were associated particularly with an independent susceptibility risk of experiencing aEARR in men or women secondary to mechanical orthodontic load. None of the previously studied genetic variants located at chromosomes 2, 4, 8, 12, and 18, also included in the present study, were found to be marginally associated with a positive or negative risk of aEARR [p value > 0.001]. In this respect, when a p value threshold was set to a conventional value of <0.05, the number of potential associations increased substantially as provided in *Supporting Information File 5*. In this line, previously studied variants at chromosomes 4, 12, and 18, i.e., *SSP1*: rs11730582 [OR: 0.54; 95%CI: 0.34–0.86; p = 0.008], *P2RX7*: rs1718119 [OR: 0.6; 95%CI: 0.36–1.01; p = 0.047], and *TNFRSF11A*: rs8086340 [OR: 0.6; 95%CI: 0.38–0.95; p = 0.024], respectively, showed marginal associations that interestingly, were found only in females, not showing the same trend for males. Importantly, all the above described marginal associations and their effects should be interpreted with caution as adjustment for multiple comparisons retrieves false discovery rate (FDR) values superior to 0.05 for all genetic targets, which does not yet allow precise discarding of the variants as not having an unequivocal modulatory effect over aEARR (Tables 2 and 3, *Supporting Information File 5*).

4. Discussion

The present study offers, for the first time, significant valuable data on genetic variants located on chromosomes X and Y that are potentially implicated in aEARR facilitated by orthodontic forces. aEARR has been defined as a permanent loss of apical dental root structure of more than 5 mm. Severe forms of EARR secondary to orthodontic forces are the least frequent type of EARR, which is more often detected as of mild or moderate degrees [7]. Thus, aEARR describes a clearly radiographically identifiable phenotype of EARR with an apical third loss of more than 5 mm that is produced within a limited period, as this is the case of a mean orthodontic treatment length of ~20 months [38]. Despite being relatively rare, aggressive phenotypes are prone to exacerbate patient morbidity and are more likely to provoke mechanical and functional disabling consequences for the tooth, potentially inducing irreversible pulp or periodontal damage and triggering an inflammatory and/or infectious process that might result in eventual tooth loss [6,39].

Therefore, from a clinical perspective, it is clearly urgent to suitably define the triggering factors that might contribute to the etiology of this complex aggressive pathology [7]. In line with this, several diagnostic and clinical factors have been previously associated with moderate degrees of EARR secondary to orthodontic forces [2,40]. Demographic factors such as age and gender, morphological factors such as root shape type or clinical factors such as treatment duration, magnitude of orthodontic forces, previous dental trauma, maxillary expansion degree, direction of tooth movement, extraction treatment, use of intermaxillary elastics, or even appliance type have been suggested [9,41–44]. Nevertheless, a significant number of controversial related results are also found in literature. Recent meta-analysis and systematic reviews have suggested a greater relevance to some of them, but these are usually based on scientific evidence with low certainty levels [9,45]. Hence, several differing clinical management protocols for aEARR have been suggested [1].

Regression analysis performed in the present study suggests that both cohorts are unlikely to be marginally influenced (p > 0.05) by some of the collected covariables. In this regard, the treatment time has been described as a risk factor influencing EARR severity within a degree-time dependent effect [44]; however, this suggestion is not supported for the case of aEARR based on the present research data. A robust risk association was not directly imputed to treatment length by itself according to comparisons between both cohorts in the current study [44–46]. It is thus plausible that some confounding factors associated with treatment length and not treatment time itself, might explain some of the differences in some cases [38,47]. In this respect, some of the lengthiest treatments are very often associated with non-conventional tooth-movement rates or uncharacteristic treatment mechanics, which might be linked with infra-diagnosed conditions or factors, i.e., this may be the case for basal differences in bone remodeling metabolism and rates, and also for differences in craniofacial muscular responses which might affect occlusal loading and even tooth micro-trauma during orthodontic treatment and may even interfere with medications such as AINES and bisphosphonates [48–50]. Although a meticulous medical history was recorded; no unquestionable certainty should be presumed from the patients' responses. Further non-compliance, round tripping movements, and movements near the bone cortex might also be associated with lower orthodontic tooth movement rates and/or extended treatment times [45,51]. Even considering these reflections, heterogeneity between study designs, ethnic differences, and the differences within phenotypic characterization might contribute to some of the observed results [17,23,52,53].

Apart from clinical-related factors, accumulating evidence supports the influence that genetic factors exert over the occurrence of post-orthodontic EARR with moderate severity. Previously published studies have provided useful evidence regarding the suggestive role of some specific genetic variants within the EARR process following candidate gene approaches [15–23]. In this respect, none of the previously studied genetic variants, also examined in the current study, showed robust statistically significant associations with aEARR [$p > 0.0001$], while a few of them (*SSP1*: rs11730582, *P2RX7*: rs1718119, *TNFRSF11A*: rs8086340) showed marginal associations [$p < 0.05$]. Interestingly, these marginal associations were found just in the female group, not showing the same trend in males, prior to and after adjustment with the clinical confounders. This might suggest a gender-specific effect. In this respect, this is the first study to perform a deep analysis regarding the influence of more than 14,000 specific genetic variants located on sexual chromosomes within an aggressive phenotype of EARR in the context of mechanical orthodontic loading. Moreover, this paper offers very valuable data regarding multiple novel putative loci and the genes potentially implicated in this type of extreme phenotype located not chromosomes X and Y, as well as at the level of other candidate genes with less power imputation located at autosomes 2, 4, 8, 12, and 18. Specifically, just two variants located at chromosome X, STAG 2 rs151184635 and RP1-30E17.2 rs55839915, where identified as the best associated SNPs [p value < 0.0001]. Interestingly, these associations showed a gender-dependent association limited to men [54,55]. We detected several statistically significant sex-specific interactions for many SNPs in the targeted genes. This sexual dimorphism is present in a vast majority of human pathologies based on genetic and hormonal differences that might modulate gene expression increase or minimize disease risk and progression [56–59]. In this regard, differences in bone remodeling rate have been described to be highly influenced by sex-specific features along with potential differences in bone mineral density and metabolism, or even hormone balance status [48,51,60]. Moreover, other gene expression factors directly linked to cytotoxic protection mechanism or related cytokine secretion might be underlying in the sex-specific differences as in other pathological entities [61–63]. Future studies should thus clarify if these results imply a potential interaction of these candidate genes with specific pathways related to gonadal steroids or additional gender-dependent mediators [64].

The top identified associations in the current study targeted rs151184635 and rs55839915 variants. RP1-30E17.2 [Ensembl: ENSG00000225689.1] contains 216,374 genetic variants with at least four splice variants. The SNP rs55839915 associated in the present study overlaps in three different transcripts being a long intergenic non-coding RNA type (lincRNA) that lacks coding potential [65]; however, lncRNAs have been described as regulators of gene expression, scaffold formation, and epigenetic control mediating within different pathological complex entities and physiological processes [66,67]. No specific underlying mechanisms have been described associated with this variant, and so, further studies should provide some potential explanatory hypothesis for aEARR. Meanwhile, STAG 2 encodes stromal antigen 2 protein, a subunit of the cohesin complex [Ensembl: ENSG00000101972 MIM: 300826]. This gene is linked to separation of chromatids during cell division, and its inactivation is associated with several types of human cancer [68]. The rs151184635 variant codes a non-coding transcript variant occurring within an intron overlapping three different transcripts at STAG2, TEX13D, and SH2D1A, all long non-coding RNA genes [69–72]. Although no direct robust functional consequence has been described in literature, SH2D1A, which is a mediator in cytolytic pathways as key activator of T- and NK cell-cytotoxicity, showed differential gene expression associated with specific immunopathologies as is the case of systemic juvenile idiopathic arthritis, associated with the potential onset of macrophage activation syndrome [73]. In this regard, extremely high overproduction of pro-inflammatory cytokines, IFN-γ, IL-1, IL-18, TNF, IL-2, IL-6, and macrophage colony-stimulating factor (M-CSF), as well as repressors such as circulating TNF receptors and IL-1ra are correlated with this macrophage-based pathology [74]. In connection with this, it has been described that prolonged mechanical strain within the periodontal ligament (PDL) around the tooth root is associated with an increase in CD68+, iNOS+ M1-like macrophages, an imbalance in the M1 > M2 ratio polarization and root resorption pathology linked to IFN-γ oversecretion by T-cells and PDL stem cells [75,76]. Whether any potential functional consequence in terms of alternative splicing or gene expression modulation might have a direct/indirect effect on the onset of aggressive phenotype of EARR, remains to be a remote hypothesis that should be explored deeply based on robust future molecular studies. Interestingly, X-chromosome activation of the vast majority of genes linked to the X chromosomes occurs in one of the two X chromosomes of any cell in women. Random or quasi-random selection of which X chromosome will remain inactivated in females follows a different process in males, which might lead to differential expression or repression of some inherited X-linked genes. This raises the possibility that expression of specific long non-coding RNAs, as should be the case for STAG2, TEX13D, or SH2D1A, might exert an influence in modulating gene expression in specific domains that escape X silencing, as occurs in other species, in a gender-dependent way. This should partially explain whether the genetic variants (rs151184635 and rs55839915) in the present study are at least marginally associated in males but not in females [77–79].

Limitations

The present study recruited a representative cohort of severely EARR affected patients that were radiographically screened by means of panoramic projections measurements >5 mm as assessed by two experienced examiners [7]. The radiographic diagnostic method used for patient assignment to affected and control groups is not exempt from limitations in terms of accuracy compared to other x-ray methods such periapical radiographs, Cone Beam Computerized Tomography (CBCT), Computerized Tomography (CT), or ex vivo methods (histological and/or histochemical) [80]. Nevertheless, panoramic radiograph assessments might produce sufficient reliability to perform absolute linear measurements when the head position is controlled in a range of 10° of the inclination in regard to the horizontal plane. Moreover, panoramic radiographs have been associated with underestimation of the tooth root lesion in mild lesions; however, this type of screening for assessing such types of aggressive phenotypes (>5 mm) is less prone to misclassification as shown by optimal reproducibility and error method results retrieved by the two experienced operators in the present study.

Secondly, although the UCM3Dg consortium database provides a unique opportunity to investigate the underlying influence of genetics over aEARR, the sample size is still relatively moderate, once the obtained results are re-analyzed, and the present design did not use an independent external cohort as a replication study due to the particularity of this aggressive phenotype. Thus, spurious associations remain a challenge and the external validity of the present findings need to be confirmed in further investigations by using different external cohorts to test the model.

Lastly, differences observed between study results in terms of the diagnostic, clinical, and genetic factors associated with EARR of different degrees are a major concern that should be addressed. With respect to this, it seems plausible that the heterogeneity found between study designs, ethnic differences [52], absence of a standardized and unique phenotypic characterization of EARR in terms of diagnostic methods and diagnostic criteria values/thresholds between studies might have contributed to some of this controversy. Nevertheless, this is a critical issue that should be clearly revisited and global consensus-based standards should be achieved in this regard to grant a next generation improvement, not only in internal validity of the studies but equally critical, in external validity of the research in the field. This should support bench-to-clinic research findings with increased certainty levels [1,81,82].

Despite all the aforementioned relevant concerns, this study offers novel and extremely valuable data regarding multiple putative loci and genes located at chromosomes X and Y potentially implicated in this type of extreme phenotype, along with other candidate genes with less imputation power at chromosomes 2, 4, 8, 12, 18, X, and Y. To the best of our knowledge, this is the first XY- chromosome-wide association study (XYWAS) to investigate sex-specific genetic effects on XY chromosomes within one of the most severe phenotypes of EARR in the context of orthodontic forces.

5. Conclusions

Multiple putative genetic variants located at chromosomes X and Y are potentially implicated in an extreme phenotype of aEARR. Particularly, STAG2 genetic variants rs151184635 and RP1-30E17.2 genetic variant rs55839915 were found to be associated with an increased risk of being afflicted with aEARR, only in men.

Supplementary Materials: The following are available online at http://www.mdpi.com/2075-4426/10/4/169/s1, Supporting Information File 1: eligibility criteria for inclusion/exclusion from the study research and diagnostic-clinical-genetic data recorded for each patient; Supporting Information File 2: full set of diagnostic, clinical, radiogrametric variables recorded for each participant; Supporting Information File 3: quality control steps performed in the genotyped sample; Supporting Information File 4: genetic variants explored in the research at chromosomes 2, 4, 8, 12, 18, X and Y; Supporting Information File 5: SNPs marginally associated with aEARR [$p < 0.05$] at chromosomes 2, 4, 8, 12, 18, X and Y.

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Supporting Information File 1. Eligibility criteria for inclusion/exclusion from the study research and diagnostic-clinical-genetic data recorded for each patient.

Eligibility criteria	**D&Cl recorded**	**Genetic factors**
Complete orthodontic treatment with fixed appliances	Age	
Caucasian origin	Sex [female; male] ¶	Chromosome X- Y [14,700 genetic variants. *See Supporting Information*
Complete root formation	Treatment time¶	
No previous history of dental trauma	Treatment type [Extraction; Non-extraction] ¶	
No systemic pathologies altering hard tissue biology	ABO Discrepancy index (DI)	Candidate SNPs previously studied within EARR [rs1800587, rs1143634, rs419598, rs315952, rs11730582, rs11573856, rs2073618, rs731236, rs1718119,rs2230912, rs7237982, rs8086340, rs17069845, rs1805034, rs12970081, rs17069898, rs4426449]
No root canal treatment therapy on measured teeth	Angle classification [Class I, Class II, Class III]	
No orthodontic retreatment	Apical displacement [Vertical; Sagittal]	
Available lateral and panoramic pre and post-treatment radiographs	Apical displacement [Vertical; Sagittal] (absolute)	
Genotyping data needed to be available	Other: Asthma medication, Angulation changes U1, Anterior elastics use	

Supporting Information File 2. Full set of diagnostic, clinical, radiogrametric variables recorded for each participant

Gender	Type of treatment [extraction, non extraction]	Apical morphology	Discrepancy index of the ABO
Age	Treatment time	Sharp morphology	Overjet
Type of anterior tooth measured	Type of extraction	Round morphology	Overbite
Endodontic treatment performed	Intraoral distalizer	Squared morphology	Anterior open bite
Maloclussion	Rapid maxillary expansion	Curve morphology	Lateral open bite
Previous tooth trauma	Slow maxillary expansion	Vertical apical absolute displacement	Crowding
Traumatized tooth	Full archwire sequence mantained	Saggital apical absolute displacement	Occlusal relationship
Asthma medication	Rebonding of studied tooth	Vertical apical displacement	Lingual posterior crossbite
Immature deglutition	Class I,II,III elastics	Saggital apical displacement	Buccal posterior crossbite
Onicophagy	Anterior elastics	Upper incisor flawring	DI cephalometric assessment
Oral breathing	Intrusive force on studied tooth	Initial Upper incisors angulation	other
Tonghe piercing	Extrusive force on studied tooth	Final Upper incisors angulation	
Smoking	Selective torsion	Tooth crown-root initial measurements	
Impacted canines	Selective inset	Tooth crown-root final measurements	
Hypoplasic tooth	Selective offset		
Microdoncy			

ABO: American Board of Orthodontics; DI: ABO Discrepancy index (from Cangialosi TJ, et al. The ABO discrepancy index: a measure of case complexity. Am J Orthod Dentofacial Orthop. 2004;125:270-278)

Supporting Information File 3. Quality control steps performed in the genotyped sample

GWAS Quality control

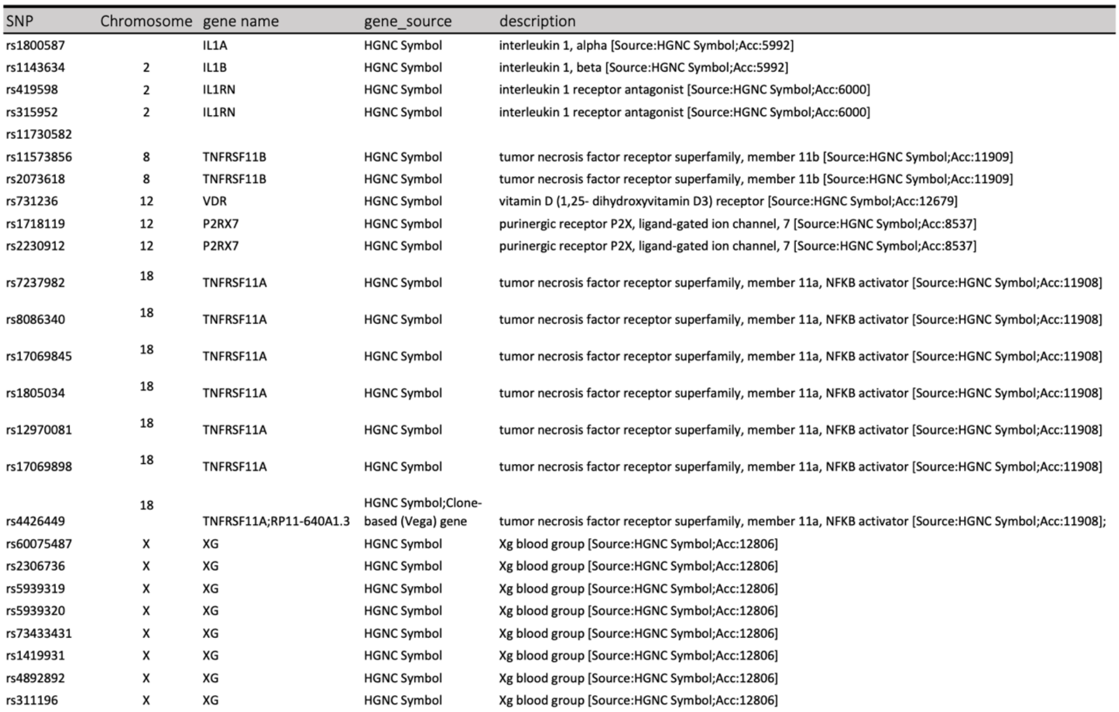
1. - Genotype missing control: The call rate of samples and SNPs analysed was higher than 0.90

2. - Minor allele frequency (MAF): We checked out the MAF in all the SNPs to ensure that enough number of the rare genotypes was present in the dataset. We only included polymorphisms with MAF ≥ 1%.

3- Cryptic relatedness: We assumed that there wasn't cryptic relatedness between study participants. Cryptic relatedness refers to the idea that some subjects of a case-control study might in fact be close relatives, thus their genotypes being not independent from the population frequencies

MAF: Minor allele frequency; SNPs: Single Nucleotide Polymorphisms

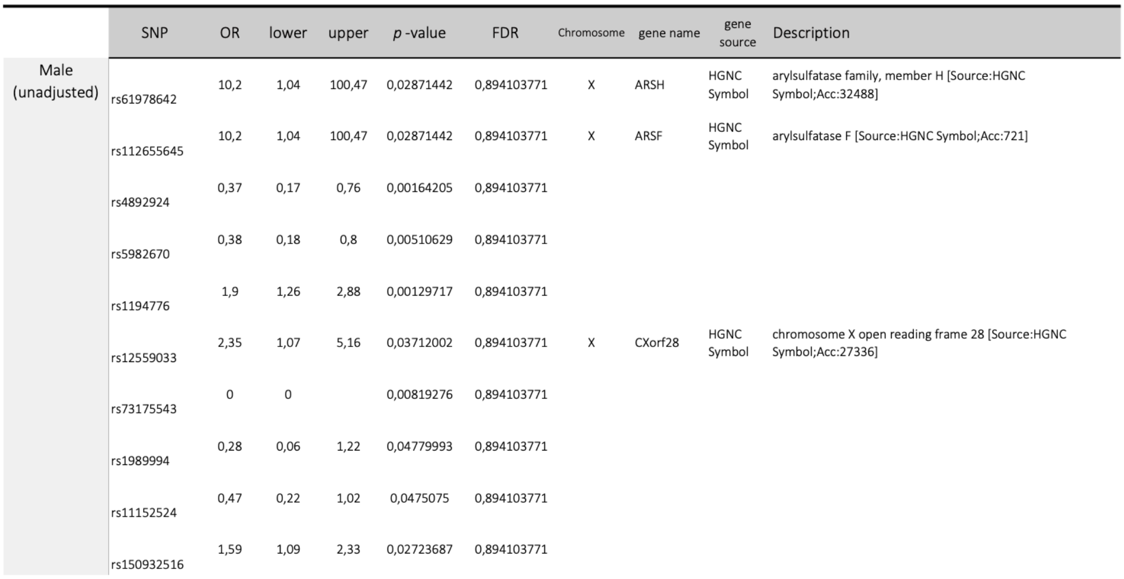
Supporting Information File 4 . Genetic variants explored in the research at chromosomes 2, 4, 8, 12, 18, X and Y.



SNP	Chromosome	gene name	gene_source	description
rs1800587		IL1A	HGNC Symbol	interleukin 1, alpha [Source:HGNC Symbol;Acc:5992]
rs1143634	2	IL1B	HGNC Symbol	interleukin 1, beta [Source:HGNC Symbol;Acc:5992]
rs419598	2	IL1RN	HGNC Symbol	interleukin 1 receptor antagonist [Source:HGNC Symbol;Acc:6000]
rs315952	2	IL1RN	HGNC Symbol	interleukin 1 receptor antagonist [Source:HGNC Symbol;Acc:6000]
rs11730582				
rs11573856	8	TNFRSF11B	HGNC Symbol	tumor necrosis factor receptor superfamily, member 11b [Source:HGNC Symbol;Acc:11909]
rs2073618	8	TNFRSF11B	HGNC Symbol	tumor necrosis factor receptor superfamily, member 11b [Source:HGNC Symbol;Acc:11909]
rs731236	12	VDR	HGNC Symbol	vitamin D (1,25- dihydroxyvitamin D3) receptor [Source:HGNC Symbol;Acc:12679]
rs1718119	12	P2RX7	HGNC Symbol	purinergic receptor P2X, ligand-gated ion channel, 7 [Source:HGNC Symbol;Acc:8537]
rs2230912	12	P2RX7	HGNC Symbol	purinergic receptor P2X, ligand-gated ion channel, 7 [Source:HGNC Symbol;Acc:8537]
rs7237982	18	TNFRSF11A	HGNC Symbol	tumor necrosis factor receptor superfamily, member 11a, NFKB activator [Source:HGNC Symbol;Acc:11908]
rs8086340	18	TNFRSF11A	HGNC Symbol	tumor necrosis factor receptor superfamily, member 11a, NFKB activator [Source:HGNC Symbol;Acc:11908]
rs17069845	18	TNFRSF11A	HGNC Symbol	tumor necrosis factor receptor superfamily, member 11a, NFKB activator [Source:HGNC Symbol;Acc:11908]
rs1805034	18	TNFRSF11A	HGNC Symbol	tumor necrosis factor receptor superfamily, member 11a, NFKB activator [Source:HGNC Symbol;Acc:11908]
rs12970081	18	TNFRSF11A	HGNC Symbol	tumor necrosis factor receptor superfamily, member 11a, NFKB activator [Source:HGNC Symbol;Acc:11908]
rs17069898	18	TNFRSF11A	HGNC Symbol	tumor necrosis factor receptor superfamily, member 11a, NFKB activator [Source:HGNC Symbol;Acc:11908]
rs4426449	18	TNFRSF11A;RP11-640A1.3	HGNC Symbol;Clone-based (Vega) gene	tumor necrosis factor receptor superfamily, member 11a, NFKB activator [Source:HGNC Symbol;Acc:11908];
rs60075487	X	XG	HGNC Symbol	Xg blood group [Source:HGNC Symbol;Acc:12806]
rs2306736	X	XG	HGNC Symbol	Xg blood group [Source:HGNC Symbol;Acc:12806]
rs5939319	X	XG	HGNC Symbol	Xg blood group [Source:HGNC Symbol;Acc:12806]
rs5939320	X	XG	HGNC Symbol	Xg blood group [Source:HGNC Symbol;Acc:12806]
rs73433431	X	XG	HGNC Symbol	Xg blood group [Source:HGNC Symbol;Acc:12806]
rs1419931	X	XG	HGNC Symbol	Xg blood group [Source:HGNC Symbol;Acc:12806]
rs4892892	X	XG	HGNC Symbol	Xg blood group [Source:HGNC Symbol;Acc:12806]
rs311196	X	XG	HGNC Symbol	Xg blood group [Source:HGNC Symbol;Acc:12806]

(480 pages) The following are available online at https://www.mdpi.com/2075-4426/10/4/169/s1,

Supporting Information File 5 . SNPs marginally associated with aEARR [$p < .05$] at chromosomes 2, 4, 8, 12, 18, X and Y.



	SNP	OR	lower	upper	*p* -value	FDR	Chromosome	gene name	gene source	Description
Male (unadjusted)	rs61978642	10,2	1,04	100,47	0,02871442	0,894103771	X	ARSH	HGNC Symbol	arylsulfatase family, member H [Source:HGNC Symbol;Acc:32488]
	rs112655645	10,2	1,04	100,47	0,02871442	0,894103771	X	ARSF	HGNC Symbol	arylsulfatase F [Source:HGNC Symbol;Acc:721]
	rs4892924	0,37	0,17	0,76	0,00164205	0,894103771				
	rs5982670	0,38	0,18	0,8	0,00510629	0,894103771				
	rs1194776	1,9	1,26	2,88	0,00129717	0,894103771				
	rs12559033	2,35	1,07	5,16	0,03712002	0,894103771	X	CXorf28	HGNC Symbol	chromosome X open reading frame 28 [Source:HGNC Symbol;Acc:27336]
	rs73175543	0	0		0,00819276	0,894103771				
	rs1989994	0,28	0,06	1,22	0,04779993	0,894103771				
	rs11152524	0,47	0,22	1,02	0,0475075	0,894103771				
	rs150932516	1,59	1,09	2,33	0,02723687	0,894103771				

(200 pages) The following are available online at https://www.mdpi.com/2075-4426/10/4/169/s1,

GWAS of post-orthodontic aggressive external apical root resorption

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Short title: GWAS and root resorption in orthodontics

Keywords: orthodontics; dentistry; resorption; fixed appliances; genetics

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ABSTRACT

Objectives: The aim of this study is to determine whether specific genetic variants might be positively/negatively associated with aggressive external apical root resorption (aEARR). Secondary aimes are to indentify any potential clinical factor that might impact this process throughout orthodontic treatment.

Material and Methods: A genome-wide association study (GWAS) was conducted in 612 participants from the UCM3Dg consortium. The study design incorporated comprehensive phenotyping based on radiographic evaluations and genotyping of 375,000 SNPs across multiple chromosomes. Rigorous quality control was applied to clinical, radiographic, and genetic data to ensure reliability. Radiographic measurements followed established scales, including the Malmgren and Brezniak methodologies, with high inter- and intra-observer agreement. Statistical analyses utilized logistic regression models adjusted for clinical and treatment-related variables to identify potential genetic associations. Ethical approval and informed consent were obtained in compliance with the Declaration of Helsinki.

Results: Genetic analysis identified 20 significant SNPs associated with aEARR ($p < 1 \times 10^{-5}$), with rs1122449 in the IRX2 gene showing the strongest association ($p < 1 \times 10^{-6}$). Other notable variants include SMAD2 (rs948604) and GLI2 (rs79617063), which are involved in key pathways related to bone remodeling and root development. Radiographic assessments confirmed high reliability (k = 1.00, ICC = 0.910) and precision (<0.04 mm error) in identifying aEARR. Logistic regression highlighted male sex, bracket rebonding, and nail-biting behavior were significantly associated with an elevated risk of aEARR, whereas the use of a 0.022” bracket slot reduced the risk.

Conclusion: This study identified the rs1122449 variant in the IRX2 gene, located on chromosome 5, as strongly associated with an increased risk of aggressive external apical root resorption (aEARR). In addition, clinical factors such as male sex, bracket rebonding, and onychophagia were also linked to a higher risk, while the use of a 0.022” bracket slot showed a protective effect.

1. INTRODUCTION

The influence of genetic variation on disease susceptibility is increasingly recognized across multiple conditions, where polymorphisms significantly impact how individuals respond to environmental and biological stressors (Virolainen, VonHandorf, Viel, Weirauch, & Kottyan, 2023). Variations in genes regulating immune response and cell adhesion have been linked to differential disease severity in inflammatory conditions such as cancer (Han et al., 2021), COVID-19 (Rotival & Quintana-Murci, 2024), or rheumatoid arthritis (Zhi et al., 2017).

In the context of orthodontics, these insights are particulary relevant to external apical root resorption (EARR), an unintended and irreversible side effect of orthodontic treatment, which raises significant concerns among clinicians due to its potential to compromise tooth stability and long-term function (van Doornik, Pijnenburg, Janssen, Ren, & Kuijpers-Jagtman, 2024). Recent findings emphasize the link between genetic disorders and pathological root resorption, expanding our understanding of how these conditions contribute to bone destruction and resorption processes (Dupre et al., 2024). This review highlighted 14 different genetic pathologies associated with root resorption, including metabolic disorders and calcium-phosphorus metabolism disorders. The authors noted that these genetic conditions often lead to an inflammatory environment conducive to the activation of osteoclasts and odontoclasts, thereby promoting resorptive processes in dental tissues. This connection between genetic predisposition and root resorption underlines the need to consider underlying genetic factors in patients presenting with severe or unexplained resorptive dental pathology.

Several factors have been documented to predispose patients to the aggressive phenotype of EARR, including patient conditions, treatment duration, specific orthodontic tooth movements (Burnheimer, Baxter, Deeley, Vieira, & Bezamat, 2024; Prasanna Arvind, Ramasamy, Subramanian, Selvaraj, & Siva, 2024; Yassir, McIntyre, & Bearn, 2021) and genetic factors, particularly some genetic variants at the level of the IL1 gene cluster, SSP1, *TNFSF11A, TNFRSF11B, WNT3A, SFRP2, LRP6, P2RX7,* and *LRP1* (Dupre et al., 2024; J. Liu, Park, Choi, Lee, & Cha, 2024; Pinheiro et al., 2021; Yassir et al., 2021). However**,** the exact role of each factor remains controversial due to inconsistencies across studies.

Despite the growing recognition of genetic influence, research on genetic susceptibility to EARR remains limited, with few genome-wide association studies (GWAS) conducted to date (Iber-Diaz et al., 2020). Existing genetic studies predominantly conclude that further research is needed with standardized diagnostic criteria for EARR and larger sample sizes to ensure more reliable findings (Dupre et al., 2024; Lee et al., 2022; J. Liu et al., 2024; Pinheiro et al., 2021; Silva et al., 2022). This highlights the need for further exploration to identify genetic markers that could help clinicians anticipate individual responses to orthodontic treatment. Such advancements would enable orthodontists to adjust force magnitude and treatment characteristics, minimizing the risk of EARR in genetically predisposed patients. This precision approach suggests a promising future for personalized orthodontics, enhancing both safety and effectiveness by aligning treatment strategies with each patient's unique genetic profile.

The aim of this study is to determine whether specific genetic variants might be positively/negatively associated with aggressive external apical root resorption (aEARR).

Secondary aimes are to indentify any potential clinical factor that might impact this process throughout orthodontic treatment.

2. MATERIAL AND METHODS

2.1. Study design

A genome-wide association study (GWAS) was conducted on aggressive apical external root resorption (aEARR), an extreme and precisely defined phenotype using participants from the UCM3D consortium (*Figure 1*). The UCM consortium database comprises a cohort of approximately 0.01 million patients aged 8 to 67, recruited from the general population across Spain between 2005 and 2024.

2.2. Sample size, Study cohorts and Ethic Statement

The sample size was estimated according to the minimum requirements for a genetic association study, taking into account the following parameters: disease prevalence of 0.01, a case-to-control ratio of approximately 1:5, an alpha error rate of 1×10^{-4} with a beta error less than 0.20, a baseline relative risk of 2.9, and a risk allele frequency of 0.25. The calculation was performed using the Genetic Power Calculation software, available as a free tool. Based on these criteria, a total of 600 participants was deemed necessary to detect a statistically significant association between the presence of the single nucleotide polymorphism (SNP) of interest and the development of an advanced stage of apical external root resorption (EARR). This sample included 100 cases representing the severely affected group and 450 controls, with an additional 3% added to account for anticipated dropouts.

The study population included 612 patients, with a case-to-control ratio of 1:5, selected from the UCM3Dg consortium database. These participants had complete radiographic, diagnostic, and clinical records, along with a full set of genetic data, and met the inclusion criteria specified in *Supporting Information File 1.*

The research received ethical approval from the Institutional Review Board (MacLochlainn, Kirby, McFadden, & Mallett) of the Clinical Hospital San Carlos, Madrid (ref:17/038-E) and informed consent was obtained from each participant. The study adhered to the ethical guidelines for human medical research as specified in the Declaration of Helsinki (World Medical, 2013).

2.3. Quality Control (QC), Data Filtering, and Extraction

A structured protocol was implemented for filtering and extracting data from the UCM3Dg and SALUDR databases. As outlined in *Supporting Information File 2*, diagnostic variables, clinical setting parameters, and radiological measurements were gathered and subjected to quality control (accession registered URI+i ref#:136-121124). Data inconsistencies in the database were addressed by isolating and correcting implausible entries across categories, as well as any misrecorded values that did not align with the expected unit type or quantitative/categorical format. Cases with extreme values were excluded, as these were likely due to data entry errors or represented non-physiological outliers rather than realistic inputs. The quality-controlled data were then prepared for further processing in the subsequent steps (*Figure 1*).

2.4. Phenotyping and Radiographic Measurements

Participants selected for final inclusion were allocated to either the affected (aEARR) or control cohorts based on orthophantomographic evaluation. The categorization was determined using Malmgren scale (Malmgren et al., 1982), with patients graded 0, 1, 2 or 3 assigned to the unaffected cohort, and those exhibiting the aggressive phenotype (aEARR) graded with score 4, assigned to the affected cohort.

Radiographic measurements were independently conducted by a single investigator, utilizing digital panoramic (P.I.) and teleradiographic (R.S.) projections, which are routinely used in clinical diagnostics and treatment planning. In orthopantomographys, the most affected and clearly visible upper incisor was selected to assess the degree of EARR. All pre- and post-treatment images were calibrated in advance, with a magnification correction factor applied in each case. Measurements were conducted using diagnostic software (Adobe Photoshop CS8, Adobe Systems Incorporated, San Jose, CA, USA), which allowed for the application of image filters to maximize precision in identifying the terminal points of the roots.

In addition, to ensure the accuracy of the Malmgren classification, supplementary measurements were also performed following the methodology outlined by Linge and Linge and subsequently modified by Brezniak. (Brezniak et al., 2004)

Apical displacement and alterations in tooth inclination were quantified by superimposing radiographic measurements on lateral radiographs, using a modified version of the approach described by Bacceti (Baccetti, McGill, Franchi, McNamara, & Tollaro).

2.5. Genotypig

Genomic DNA was isolated from saliva samples following the protocol provided by the manufacturer (prepIT•L2P, DNA Genotek, Ottawa, ON, Canada). The extracted DNA was evaluated for purity and integrity, ensuring OD260/OD280 ratios of 1.8–2.0 and OD260/OD230 values above 1.5, as well as confirming that 90% of the DNA fragments were greater than 10 Kb in size on a 1% agarose gel. Sample quality control was conducted using the Agena Bioscience MassARRAY platform with iPLEX GOLD technology, enabling the exclusion of low-quality samples.

The genotyping of DNA samples was carried out with the high-throughput Axiom platform, using the GeneTitan® MC Instrument and Axiom Genome-Wide Human Assay technology (CeGen). This approach has a strong validation record in the literature. Genotype clustering and calling were performed using Axiom Analysis Suite 4.0 software. Subsequent quality control steps included checks for sample stratification, missing genotype data, monomorphic SNPs, and minor allele frequencies (*Supporting Information File 3*). To meet the study's objectives, we selected 375.000 SNPs located on the X and Y-chromosomes, along with additional candidate genes on chromosomes 1,2,3,4,5,6,8,10,12,14,15,18,19 for further analysis.

2.6. Statistics

2.6.1. Overall Statistical Analysis of Clinical and Radiological Variables

A descriptive analysis was conducted for both quantitative and categorical variables based on diagnostic and treatment-related factors, encompassing the mean, standard deviation, ranges, frequencies, and distributions. Initially, a simple backward conditional binary logistic regression was applied using all variables, followed by a multiple model including only the statiscally significant variables ($p<0.05$) to evaluate the influence of specific diagnostic and treatment parameters on the observed apical external root resorption (aEARR) process, with odds ratios (OR) and 95% confidence intervals calculated. Data analysis was carried out using SPSS software (version 22.0; LEAD Technologies, Chicago, IL, USA), with statistical significance established at $p < 0.05$.

2.6.2. Genetic Association Tests

To identify the most suitable SNP candidates, a logistic regression model was employed, adjusting for treatment type, total treatment duration, apical displacement and gender, while assuming a log-additive genetic model. Statistical analyses were conducted using R software (R Core Team, Vienna, Austria, version 3.6.1).

2.6.3. Reliability and Accuracy of the Measurement Method

To minimize inter-observer variation, each operator performed measurements on a specific type of radiograph: an experienced operator (P.I) conducted all panoramic measurements, while another operator (R.S) handled the lateral teleradiographic measurements. Each operator underwent calibration specifically for their assigned type of radiograph to ensure consistency and accuracy within their modality. The results obtained from these measurements were then compared with findings from previous studies in this field to verify their coherence with established data.

To assess intra-observer reliability in patient classification (EARR-affected and non-affected), both a visual method (Malmgren et al., 1982) and a quantitative measurement method (Brezniak et al.) were applied to each radiograph. Concordance tests, including the intraclass correlation coefficient (ICC) were conducted to determine the level of agreement between the Malgrem scores and the EARR measurements in millimeters obtained with the Brezniak method. The examiner repeated the classification measurements on a subset of 200 patients after a two-month interval. Concordance was evaluated using the kappa coefficient.

Additionally, to determine the method error for quantitative radiographic measurements, double measurements were taken from 20 randomly selected subjects with a 20-day interval between measurements. A paired Student's t-test was applied to assess the significance of any differences, with non-significant results indicating concordance between repeated measurements. The intraclass correlation coefficient (ICC) for absolute agreement was also calculated to assess both intra- and inter-observer reliability. The accuracy of measurement was determined using the formula: SE=2n∑d2, where d represents the difference between paired measurements and nnn is the number of paired measurements.

3. RESULTS

3.1. Phenotyping: Reliability of the Radiographic Measurement Methods and the Associated Errors

aEARR was assessed using radiographic measurements, setting a Score of 3 or 4 (Malmgren et al., 1982) to define significant root resorption. To ensure reliability in classification, concordance between EARR-affected and non-affected groups was evaluated using the kappa index, yielding optimal agreement (k = 1.00). Concordance between methods was assessed through the intraclass correlation coefficient (ICC=0.910) confirming a high level of agreement.

Each examiner also underwent an intra-observer reliability assessment on their specific type of radiograph. The ICC was calculated for both examiners' intra-observer reliability, with no statistically significant differences observed between replicated assessments ($p > 0.05$), indicating consistent measurements over time.

Lastly, to ensure consistency with established data, the comparison with findings from previous studies in this field, confirmed that our measurements align closely with those reported in the literature, further supporting the validity of our approach. The method error for measurements, following the described methodology, was calculated to be below <0.04 mm, reinforcing the precision of the measurements conducted in this study.

3.2. Sample Characteristics, Description and Analysis of aEARR Risk Associated with Clinical Features

The sampling and research methodology are outlined in the flow chart (Figure 1), detailing the filtering steps applied. The study sample comprised a cohort of 612 patients with relatively homogenous diagnostic profiles as summarized in *Table* 1. The mean age of patients in the aEARR-affected cohort was approximately 14.5 ± 5 years, while the control group had a mean age of around 17.1 ± 10 years, with a balanced distribution of sexes between the groups. The American Board of Orthodontics (ABO) discrepancy index (Cangialosi et al., 2004) was comparable across both cohorts, with a mean score of ~15 ± 8. Treatment duration averaged around 32 ± 12 months in the aEARR group and 28 ± 13 months in the control group. It should be noted that *Table 1* includes some missing values, , which were not consistently reported by orthodontists or patients. These missing values were excluded from the statistical analysis, which may result in minor discrepancies in percentage totals.

The logistic regression analysis highlighted several clinical factors associated with an elevated risk of aEARR. In the simple model, 13 variables were initially identified as significant risk factors for aggressive external apical root resorption. However, in the multiple model, only four variables remained statistically significant. Male sex was associated with a notably higher risk of aEARR compared to female sex (OR = 2.32, 95% CI: 1.10–4.90, p = 0.027). Regarding treatment-related factors, the use of a 0.022" bracket slot was inversely associated with aEARR risk, suggesting a protective effect (OR = 0.12, 95% CI: 0.03–0.54, p = 0.006). In contrast, bracket rebonding was linked to a substantial increase in aEARR risk, with patients requiring rebonding showing over a threefold risk elevation (OR = 3.28, 95% CI: 1.47–7.31, p = 0.004). Furthermore, the behavioral factor of onychophagia (nail-biting) was also significantly associated with a higher risk of aEARR (OR = 2.85, 95% CI: 1.22–6.63, p = 0.015).

3.3. Confounding factors related to EARR. Genotype Distributions and Analysis of aEARR Risk Associated with Genetic Variants at Multiple Putative Loci

In addition to considering diagnostic and treatment-related co-variables, patients were genotyped for novel target genetic variants, as well as for other SNPs previously associated with EARR risk. These genetic markers were distributed across several chromosomes, including 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 15, 18, 19, X, and Y (*Supporting Information File 3*) and a multilevel model with multiple ethnic strata was employed.

Upon analyzing the entire cohort, a total of 20 genetic variants were identified as significantly associated with an increased risk of aggressive external apical root resorption (aEARR) ($p < 1 \times 10^{-5}$). Among these, the most notable finding was rs1122449, a single nucleotide polymorphism located in the *IRX2* gene on chromosome 5. This variant was the only one to demonstrate a p-value $< 1 \times 10^{-6}$ (8.31×10^{-6}). The *IRX2* gene belongs to the Iroquois homeobox family, members of this family appear to play multiple roles during pattern formation of vertebrate embryos [OMIM, Apr 2004] *(Tables 2 and 3).*

Additionally, among the remaining 19 genetic variants associated with aEARR ($p < 1 \times 10^{-5}$) *SMAD2* (rs948604) and *GLI2* (rs79617063) stand out due to their specific involvement in critical biological pathways related to bone remodeling and development. The protein encoded by *SMAD2* gene belongs to a family of proteins that are signal transducers and transcriptional modulators that mediate multiple signaling pathways. This protein mediates the signal of the transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation [RefSeq, May 2012]. While *GLI2* gen encodes a protein characterized as transcription factor which bind DNA through zinc finger motifs. It is also thought to play a role during embryogenesis. The encoded protein is associated with several phenotypes- Greig cephalopolysyndactyly syndrome, Pallister-Hall syndrome, preaxial polydactyly type IV, postaxial polydactyly types A1 and B. [RefSeq, Jul 2008].

The Manhattan plot generated from the analysis highlights the significant loci associated with EARR, with the several SNPs surpassing the significance threshold, thereby marking regions of interest for further investigation into their potential biological roles (*Figure 2*).

4. DISCUSSION

In this study, a rigorous approach to classify aggressive external apical root resorption (aEARR) was adopted, based on a more stringent threshold. Specifically, the EARR affected group was defined as extreme resorption score of Malgrem, focusing on a phenotype that reflects a more severe and clinically significant form of root resorption. This approach differs from the majority of studies in the literature, which commonly set a lower threshold (Iglesias-Linares et al., 2014; Lee et al., 2022; Linhartova, Cernochova, & Izakovicova Holla, 2013). By concentrating on a more aggressive phenotype, our study aims to reduce the influence of minor resorptive changes that may lack clinical impact and to provide a clearer distinction between mild and severe resorption.

Another notable strength of this study is the sample size. Studies examining genetic and clinical factors influencing EARR include sample sizes ranging from as few as 40 patients (George et al., 2020) to 377 (Fontana et al., 2012), with one study of our group reporting 462 patients (Iber-Diaz et al., 2020). These relatively small sample sizes limit statistical power and generalizability of their findings. By contrast, our study comprised a sample of 612 patients, providing a more comprehensive dataset to explore associations and identify significant risk factors. This larger sample size enhances the reliability of our results and allows for the detection of subtler effects that might be overlooked in smaller studies, offering stronger evidence for clinical translation.

The genes identified in this study have not been previously linked directly to external apical root resorption (EARR). This lack of prior association may be explained by the aggressive phenotype of EARR specifically selected for in our cohort, which could highlight genetic variants not captured in studies of milder or generalized forms of root resorption, and also because of the strength of the sample size explained before. Among the identified variants, the SNP rs1122449, located in the *IRX2* gene, displayed the strongest association with EARR. *IRX2* is well-recognized for its critical role in embryonic development and tissue patterning, as well as its involvement in promoting cell proliferation and invasion. Notably, mRNA expression of *IRX2* has been shown to be upregulated in osteosarcoma, the most common type of malignant bone tumor. This gene is a key regulator of the *PI3K/AKT* signaling pathway, which governs various processes essential to tumor progression, including cell proliferation, differentiation, migration, invasion, apoptosis, and angiogenesis. (T. Liu et al., 2015). Consequently, it is plausible that *IRX2* plays a role in the resorptive processes affecting the alveolar bone and surrounding structures during orthodontic-induced stress. This highlights the potential involvement of *IRX2* in mediating the aggressive external apical root resorption (aEARR) observed in this study.

SMAD2 (rs948604), among the 20 genetic variants associated with aEARR identified in this study, emerges as a key regulator within the *TGF-β* signaling pathway, playing an important role in both root development and bone remodeling. It regulates the expression of osteoprotegerin (OPG), an essential inhibitor of osteoclastogenesis, protecting cementum and preventing excessive root resorption during orthodontic treatment (Wang et al., 2023). Experimental evidence shows that stimulation of cementoblasts with *TGF-β1* enhances OPG secretion, reducing osteoclastic activity and limiting root resorption (Mo et al., 2024). These findings suggest that genetic variants in *SMAD2* influence root resorption by modulating *TGF-β1* signaling, highlighting its therapeutic potential in preventing aEARR.

The rs79617063 SNP in the *GLI2* gene, identified in this study, also underscores its potential role in aEARR through the Sonic Hedgehog (SHH) signaling pathway (Maranon-Vasquez et al., 2019). *GLI2* regulates epithelial-mesenchymal interactions critical for craniofacial development, bone remodeling, and has been associated with tooth agenesis and abnormal craniofacial phenotypes (Grachtchouk et al., 2021; Maranon-Vasquez et al., 2019). Furthermore, *GLI2* promotes osteoclast activation via PTHrP expression, contributing to pathological bone resorption in oral squamous cell carcinoma (Cannonier, Gonzales, Ely, Guelcher, & Sterling, 2016). These findings suggest that *GLI2* influences both normal and pathological bone remodeling, potentially exacerbating root resorption under orthodontic stress.

On the other hand, the logistic regression analysis identified four clinical significant factors associated with an increased risk of aggressive external apical root resorption (aEARR) in the multiple model. The association between male sex and a higher risk of aEARR has been reported in other studies, suggesting that alterations in plasma levels of estrogen, progesterone, and testosterone hormones can influence the rate of orthodontic tooth movement and root resorption (Seifi, Ezzati, Saedi, & Hedayati, 2015). Additionally, a recent GWAS identified genetic differences in susceptibility to root resorption were linked to sex chromosomes, specifically on the X and Y chromosomes (Iber-Diaz et al., 2020). Their findings showed that male patients, due to genetic factors associated with these chromosomes, may have an inherent predisposition to aEARR. This genetic predisposition reinforces the notion that sex differences play a critical role in orthodontic treatment outcomes, possibly linked to both hormonal influences and genetic variations that affect bone density and response to orthodontic forces.

The protective effect of using a 0.022” bracket slot is particularly noteworthy in our findings. Wider slot sizes could reduce friction during orthodontic movements, potentially minimizing the inflammatory response that contributes to root resorption and may serve as a modifiable factor in managing resorption risk. In contrast, two other studies have found no significant differences between 0.018” and 0.022” slots (El-Angbawi, Yassir, McIntyre, Revie, & Bearn, 2019; Vieira et al., 2018). Currently, evidence remains insufficient regarding the influence of different bracket slot sizes on the overall effectiveness of orthodontic treatment.

Bracket rebonding emerged as a substantial risk factor for aEARR. Rebonding involves the removal and reattachment of brackets, often in a new position to refine tooth alignment. The process of debonding, can compromise tooth integrity, leading to microfractures in the enamel and increased mechanical stress on the underlying tooth structures (Holberg, Winterhalder, Holberg, Wichelhaus, & Rudzki-Janson, 2014). These microfractures weaken the tooth surface, making it more susceptible to additional damage during subsequent orthodontic forces. Moreover, the attachment of brackets in a new position typically requires the use of stiffer archwires, resulting in higher orthodontic forces that accelerate tooth movement. This, combined with the overcorrection of tooth positions, induces greater tension and compression in the periodontal ligament (PDL), further amplifying the resorptive cycle. This aligns with our findings, suggesting that minimizing bracket rebonding events could be beneficial in reducing aEARR risk.

Behavioral factors such as nail-biting have the potential to introduce additional stress on teeth and supporting structures, potentially exacerbating root resorption (Pastro et al., 2018). In this study, onychophagia was associated with an increased risk of aEARR, possibly due to the microtraumas inflicted on the upper incisors. The repetitive and unintentional pressure exerted by nail-biting might predispose patients to a heightened resorptive response. Although no studies have directly established a relationship between nail-biting and the aggressive EARR phenotype, such an association has been suggested. Further research is necessary to explore these potential connections, confirm the findings, and refine best practices for managing aEARR risk in patients with parafunctional habits.

The SNPs identified in this study, along with the clinical variables, should be considered in the analysis of future studies to better understand the multifactorial nature of aggressive external apical root resorption (aEARR). These findings emphasize the importance of integrating both genetic and clinical risk factors in future research. To achieve consistent and reliable results in this field, future studies should focus on larger, well-characterized cohorts and adopt standardized methodologies, specifically targeting aggressive aEARR phenotypes. Such efforts are essential to refine our understanding of the mechanisms underlying aEARR and to develop strategies for personalized risk management in orthodontic treatment.

5. CONCLUSION

This study identified the rs1122449 variant in the IRX2 gene, located on chromosome 5, as strongly associated with an increased risk of aggressive external apical root resorption (aEARR). In addition, clinical factors such as male sex, bracket rebonding, and onychophagia were also linked to a higher risk, while the use of a 0.022” bracket slot showed a protective effect.

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FIGURE CAPTIONS:

Figure 1. Flow diagram of the filtering process and genome-wide association study

Figure 2. Manhattan plot

TABLE CAPTIONS:

Table 1. Population demograhics, diagnostic and clinical characteristics of the included patients.

Table 2. Lead genetic variants associated with aggresive external apical root resorption.

Table 3. Lead genes description, type and information.

Supporting Information:

Supporting Information File 1. Elegibility criteria for inclusion/exclusion from the study research and genetic data recorded for each patient

Supporting Information File 2. Diagnostic, clinical and radiographic variables recorded for each patient.

Supporting Information File 3. Quality control steps performed in the genotyped sample.

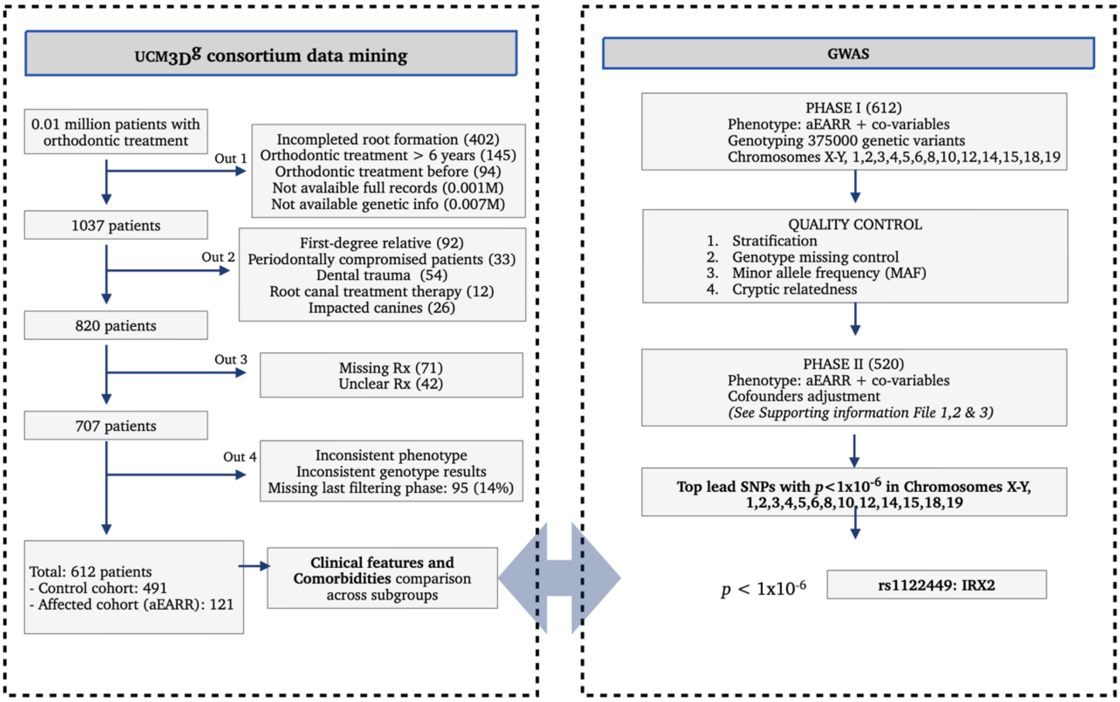

Figure 1. Flow diagram of the filtering process and genome-wide association study

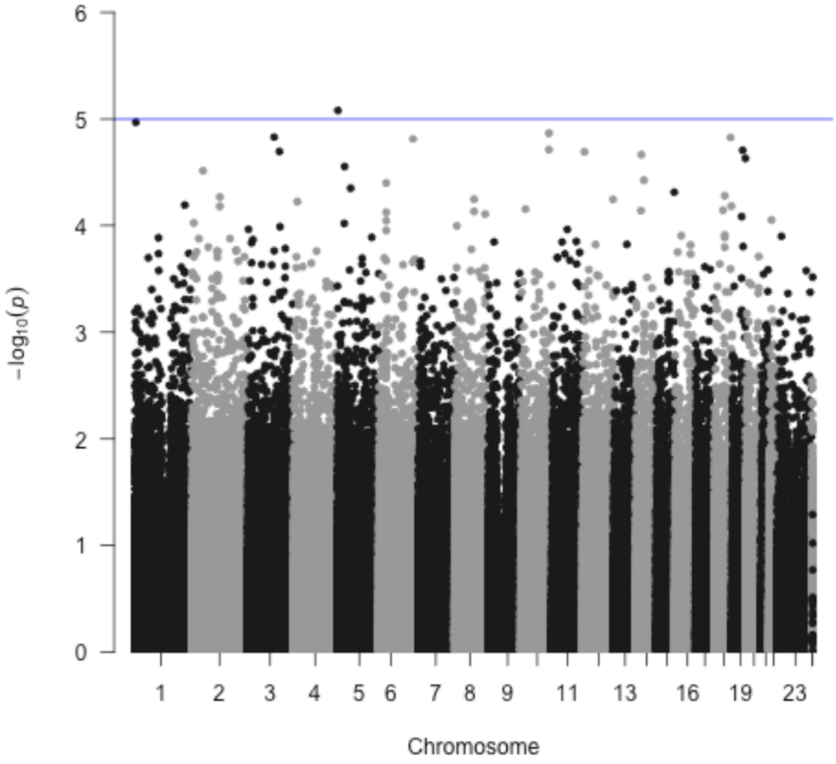

Figure 2. Manhattan plot

Table 1 **. Population demograhics, diagnostic and clinical characteristics of the included patients**

Diagnostic and clinical factors	**aEARR Cohort (n=121)**	**Control group (n=491)**	Simple model				Multiple model			
			OR	**95% CI** **Min.**	**95% CI** **Max.**	***p* value**	**OR**	**95% CI** **Min.**	**95% CI** **Max.**	***p* value**
Mean age [years (±SD)]	14.59 (5.84)	17.16 (10.08)	0.96	0.93	0.99	**0.010***	0.98	0.94	1.03	0.529
Sex										
Female [n (%)]	59 (48.8)	290 (59.1)	1				1			
Male [n (%)]	62 (51.2)	201 (40.9)	1.52	1.02	2.26	**0.041***	2.32	1.10	4.90	**0.027***
Ethnic-demographic origin										
East Europe [n (%)]	96 (79.3)	371 (75.6)	1			0.803				
West Europe [n (%)]	1 (0.8)	11 (2.2)	0.97	0.41	2.28					
Latin America [n (%)]	7 (5.8)	28 (5.7)	0.35	0.05	2.76	0.319				
North Africa [n (%)]	0 (0)	5 (1)	_	_	_	_				
Skeletal maloclussion										
Class I [n (%)]	42 (34.7)	175 (35.6)	1			0.129				
Class II [n (%)]	53 (43.8)	245 (49.9)	0.90	0.58	1.41	0.650				
Class III [n (%)]	25 (20.7)	66 (13.4)	1.58	0.89	2.79	0.117				
Dental maloclussion										
Class I [n (%)]	41 (33.9)	172 (35)	1			**0.026***	1			0.370
Class II/1 [n (%)]	33 (27.3)	172 (35)	0.81	0.49	1.33	0.399	0.49	0.20	1.17	0.109
Class II/2 [n (%)]	28 (23.1)	78 (15.9)	1.99	1.03	3.83	**0.040***	0.41	0.09	1.83	0.244
Class III [n (%)]	18 (14.9)	38 (7.7)	1.51	0.87	2.61	0.145	0.54	0.18	1.56	0.252
Treatment time [months (±SD)]	31.83 (12.03)	27.98 (12.92)	1.02	1.00	1.03	**0.022***	1.02	0.99	1.05	0.154
Tretament type										
Fixed appliance	120 (99.2)	455 (92.7)	1							
Aligners	1 (0.8)	36 (7.3)	0.11	0.01	0.77	**0.027***				
Treatment										
Extraction[n (%)]	15 (12.4)	56 (11.4)	1							
Non-extraction[n (%)]	105 (86.8)	431 (87.8)	0.91	0.50	1.67	0.760				
Treatment phases										
2 phases[n (%)]	9 (7.4)	32 (6.5)	1							
1 phases[n (%)]	23 (19)	115 (23.4)	0.71	0.30	1.69	0.440				
Discrepancy index ABO*[score (±SD)]	14.97 (8.64)	14.97 (8.20)	1.00	0.98	1.03	0.997				
Bracket slot										
0.018" [n (%)]	108 (89.3)	359 (73.1)	1				1			
0.022" [n (%)]	11 (9.1)	90 (18.3)	0.41	0.21	0.79	**0.008***	0.12	0.03	0.54	**0.006***
Movement devices										
Distalizer appliance [n (%)]	3 (2.5)	24 (4.9)	0.50	0.15	1.67	0.257				
Maxillary expansion [n (%)]	20 (16.5)	51 (10.4)	1.71	0.98	3.00	0.061	2.12	0.74	6.08	0.162
Dental expansion [n (%)]	21 (17.4)	72 (14.7)	1.22	0.71	2.07	0.471				
Other factors related with treatment										
Incorrect archwire sequence [n (%)]	54 (44.6)	222 (45.2)	1.02	0.69	1.53	0.908				
Bracket rebonding [n (%)]	70 (57.9)	211 (43)	1.83	1.22	2.75	**0.003***	3.28	1.47	7.31	**0.004***
Class elastics [n (%)]	119 (98.3)	459 (93.5)	7.26	0.98	53.9	0.053	1.18	0.11	12.9	0.890
Anterior elastics [n (%)]	33 (27.3)	132 (26.9)	1.01	0.65	1.59	0.950				
Inset [n (%)]	11 (9.1)	44 (9)	1.01	0.51	2.03	0.969				
Offset [n (%)]	5 (4.1)	36 (7.3)	0.54	0.21	1.42	0.212				
Intrusion [n (%)]	37 (30.6)	129 (26.3)	1.23	0.80	1.91	0.346				
Extrusion [n (%)]	20 (16.5)	103 (21)	0.74	0.44	1.26	0.271				
Torque [n (%)]	37 (30.6)	152 (31)	0.98	0.64	1.51	0.925				
Apical displacement										
Vertical [mm (±SD)]	-5.81 (4.50)	-7.21 (6.11)	1.05	1.00	1.10	**0.039***	1.08	0.99	1.16	0.070
Sagital [mm (±SD)]	-4.75 (3.91)	-4.68 (4.02)	0.99	0.93	1.07	0.895				
Vertical [mm (±SD)] (absolute)	3.53 (4.32)	3.98 (5.39)	0.98	0.89	1.09	0.704				
Sagital [mm (±SD)] (absolute)	4.61 (3.86)	4.21 (3.31)	1.03	0.95	1.12	0.450				
Dif. Angle [º (±SD)]	14.31 (102.84)	2.73 (11.77)	1.02	1.00	1.04	**0.049***	1.01	0.99	1.02	0.383
Teeth with the most EARR selected										
Upper central incisor [n (%)]	49 (40.5)	214 (43.6)	1							
Upper lateral incisor [n (%)]	72 (59.5)	277 (56.4)	1.14	0.76	1.70	0.539				
Apical morphology										
Curved [n (%)]	41 (33.9)	133 (27.1)	1			0.148				
Rounded [n (%)]	31 (25.6)	155 (31.6)	0.65	0.39	1.09	0.103				
Pointed [n (%)]	46 (38)	170 (34.6)	0.88	0.54	1.42	0.593				
Square [n (%)]	3 (2.5)	31 (6.3)	0.31	0.09	1.08	0.066				
Other factors										
Hypoplasia [n (%)]	11 (9.1)	40 (8.1)	1.06	0.53	2.13	0.871				
Microdontia [n (%)]	29 (24)	70 (14.3)	1.78	1.09	2.89	**0.021***	1.78	0.77	4.13	0.178
Asthma medication [n (%)]	4 (3.3)	16 (3.3)	1.01	0.33	3.08	0.985				
Onychophagia [n (%)]	21 (17.4)	60 (12.2)	2.17	1.20	3.93	**0.011***	2.85	1.22	6.63	**0.015***
EARR [mm (±SD)]	5.43 (1.82)	1.41 (1.07)	12.1	6.91	21.1	**<0.001***				

aEARR: aggresive external apical root resorption. *p <0.05

Table 2 **. Lead genetic variants associated with aggresive external apical root resorption**

SNP	Gene name	Chr	*p* value ª	MAF
rs1122449	*IRX2*	5	8,31E-06	0,072
rs4845804		1	1,06901E-05	0,092
rs11199464	*LINC02930*	10	1,3546E-05	1,442
rs35999150	*ZBTB20*	3	1,47589E-05	1,224
rs9948277	*NETO1*	18	1,48987E-05	1,018
rs72563846	*SASH1*	6	1,53914E-05	1,503
rs11199512	*LINC02930*	10	1,94453E-05	1,443
rs569450218	*TOMM40*	19	1,97505E-05	0,715
rs917934	*LOC105374126*	3	2,02597E-05	1,445
rs142150232	*CLSTN3*	12	2,04064E-05	0,199
rs17637448		14	2,1705E-05	0,438
rs185700073	*USP29*	19	2,35044E-05	1,096
rs34808837	*PDZD2*	5	2,80699E-05	0,529
rs6545039		2	3,07479E-05	0,743
rs17127526		14	3,76655E-05	0,526
rs3134604	*PPT2;PRRT1;LOC100507547;PPT2-EGFL8*	6	4,00507E-05	0,517
rs895377	*RAB3C*	5	4,4786E-05	0,745
rs76796473	*PGPEP1L*	15	4,87395E-05	1,262
rs948604	*SMAD2*	18	5,26912E-05	0,679
rs79617063	*GLI2*	2	5,41977E-05	1,318

ª Selected p value threshold $p < 1 \times 10^{-5}$; MAF: Minor Allele Frecuency; Co-variables used for adjustment: Sex, Treatment time, Treatment type and appical displacement

***Table 3*. Lead genes description, type and information**

SNP	Chr	Gene name	Gene description	Gene type	Gene information
rs1122449	5	*IRX2*	iroquois homeobox 2	protein coding	IRX2 is a member of the Iroquois homeobox gene family. Members of this family appear to play multiple roles during pattern formation of vertebrate embryos.[supplied by OMIM, Apr 2004]
rs4845804	1				
rs11199464	10	*LINC02930*	long intergenic non-protein coding RNA 2930	ncRNA	
rs35999150	3	*ZBTB20*	zinc finger and BTB domain containing 20	protein coding	This gene, which was initially designated as dendritic cell-derived BTB/POZ zinc finger (DPZF), belongs to a family of transcription factors with an N-terminal BTB/POZ domain and a C-terminal DNA-bindng zinc finger domain. The BTB/POZ domain is a hydrophobic region of approximately 120 aa which mediates association with other BTB/POZ domain-containing proteins. This gene acts as a transcriptional repressor and plays a role in many processes including neurogenesis, glucose homeostasis, and postnatal growth. Mutations in this gene have been associated with Primrose syndrome as well as the 3q13.31 microdeletion syndrome. Alternative splicing results in multiple transcript variants encoding distinct isoforms. [provided by RefSeq, Feb 2017]
rs9948277	18	*NETO1*	neuropilin and tolloid like 1	protein coding	This gene encodes a transmembrane protein containing two extracellular CUB domains followed by a low-density lipoprotein class A (LDLa) domain. This protein is thought to play a critical role in spatial learning and memory by regulating the function of synaptic N-methyl-D-aspartic acid receptor complexes in the hippocampus. Alternatively spliced transcript variants encoding multiple isoforms have been observed for this gene. [provided by RefSeq, Aug 2017]
rs72563846	6	*SASH1*	SAM and SH3 domain containing 1	protein coding	This gene encodes a scaffold protein involved in the TLR4 signaling pathway that may stimulate cytokine production and endothelial cell migration in response to invading pathogens. The encoded protein has also been described as a potential tumor suppressor that may negatively regulate proliferation, apoptosis, and invasion of cancer cells, and reduced expression of this gene has been observed in multiple human cancers. Mutations in this gene may be associated with abnormal skin pigmentation in human patients. [provided by RefSeq, Oct 2016]
rs11199512	10	*LINC02930*	long intergenic non-protein coding RNA 2930		
rs569450218	19	*TOMM40*	translocase of outer mitochondrial membrane 40	protein coding	The protein encoded by this gene is localized in the outer membrane of the mitochondria. It is the channel-forming subunit of the translocase of the mitochondrial outer membrane (TOM) complex that is essential for import of protein precursors into mitochondria. Alternatively spliced transcript variants have been found for this gene. [provided by RefSeq, Aug 2015]
rs917934	3	*LOC105374126*		ncRNA	
rs142150232	12	*CLSTN3*	calsyntenin 3	protein coding	Enables cell-cell adhesion mediator activity and neurexin family protein binding activity. Involved in L-ascorbic acid metabolic process and regulation of synapse assembly. Predicted to be located in several cellular components, including Golgi membrane; dendrite; and postsynaptic density. Predicted to be part of protein-containing complex. Predicted to be active in several cellular components, including GABA-ergic synapse; lipid droplet; and postsynaptic density membrane. [provided by Alliance of Genome Resources, Nov 2024]
rs17637448	14				
rs185700073	19	*USP29*	ubiquitin specific peptidase 29	protein coding	Predicted to enable cysteine-type deubiquitinase activity and cysteine-type endopeptidase activity. Predicted to be involved in several processes, including G1/S transition of mitotic cell cycle; positive regulation of type I interferon-mediated signaling pathway; and protein K48-linked deubiquitination. Predicted to be located in perinuclear region of cytoplasm. Predicted to be active in cytosol and nucleus. [provided by Alliance of Genome Resources, Nov 2024]
rs34808837	5	*PDZD2*	PDZ domain containing 2	protein coding	The protein encoded by this gene contains six PDZ domains and shares sequence similarity with pro-interleukin-16 (pro-IL-16). Like pro-IL-16, the encoded protein localizes to the endoplasmic reticulum and is thought to be cleaved by a caspase to produce a secreted peptide containing two PDZ domains. In addition, this gene is upregulated in primary prostate tumors and may be involved in the early stages of prostate tumorigenesis. [provided by RefSeq, Dec 2015]
rs6545039	2				
rs17127526	14				
rs3134604	6	*PPT2*	palmitoyl-protein thioesterase 2	protein coding	This gene encodes a member of the palmitoyl-protein thioesterase family. The encoded glycosylated lysosomal protein has palmitoyl-CoA hydrolase activity in vitro, but does not hydrolyze palmitate from cysteine residues in proteins. Alternative splicing results in multiple transcript variants. Read-through transcription also exists between this gene and the downstream EGFL8 (EGF-like-domain, multiple 8) gene. [provided by RefSeq, Feb 2011]
		PRRT1	proline rich transmembrane protein 1	protein coding	Enables identical protein binding activity. Predicted to be involved in several processes, including long-term synaptic depression; protein localization to cell surface; and regulation of AMPA receptor activity. Predicted to act upstream of or within several processes, including learning or memory; long-term synaptic potentiation; and synapse organization. Predicted to be located in plasma membrane and synaptic vesicle membrane. Predicted to be active in glutamatergic synapse and postsynaptic density membrane. [provided by Alliance of Genome Resources, Nov 2024]
		LOC100507547		ncRNA	
		PPT2-EGFL8	PPT2-EGFL8 readthrough (NMD candidate)	ncRNA	This locus represents naturally occurring read-through transcription between the neighboring PPT2 (palmitoyl-protein thioesterase 2) and EGFL8 (EGF-like-domain, multiple 8) genes located in the major histocompatibility complex class III region of chromosome 6. The read-through transcript is a candidate for nonsense-mediated mRNA decay (NMD), and is thus unlikely to produce a protein product. [provided by RefSeq, Feb 2011]
rs895377	5	*RAB3C*	RAB3C, member RAS oncogene family	protein coding	This gene is a member of the RAS oncogene family and encodes a small GTPase. Other similar small GTPases are known to be involved in vesicle trafficking, and the encoded protein was shown to play a role in recycling phagocytosed MHC class 1 complexes to the cell surface. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Dec 2015]
rs76796473	15	*PGPEP1L*	pyroglutamyl-peptidase I like	protein coding	Predicted to enable pyroglutamyl-peptidase activity. Predicted to be involved in proteolysis. Predicted to be located in cytosol. [provided by Alliance of Genome Resources, Nov 2024]
rs948604	18	*SMAD2*	SMAD family member 2	protein coding	The protein encoded by this gene belongs to the SMAD, a family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the C. elegans gene Sma. SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways. This protein mediates the signal of the transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation. This protein is recruited to the TGF-beta receptors through its interaction with the SMAD anchor for receptor activation (SARA) protein. In response to TGF-beta signal, this protein is phosphorylated by the TGF-beta receptors. The phosphorylation induces the dissociation of this protein with SARA and the association with the family member SMAD4. The association with SMAD4 is important for the translocation of this protein into the nucleus, where it binds to target promoters and forms a transcription repressor complex with other cofactors. This protein can also be phosphorylated by activin type 1 receptor kinase, and mediates the signal from the activin. Alternatively spliced transcript variants have been observed for this gene. [provided by RefSeq, May 2012]
rs79617063	2	*GLI2*	GLI family zinc finger 2	protein coding	This gene encodes a protein which belongs to the C2H2-type zinc finger protein subclass of the Gli family. Members of this subclass are characterized as transcription factors which bind DNA through zinc finger motifs. These motifs contain conserved H-C links. Gli family zinc finger proteins are mediators of Sonic hedgehog (Shh) signaling and they are implicated as potent oncogenes in the embryonal carcinoma cell. The protein encoded by this gene localizes to the cytoplasm and activates patched Drosophila homolog (PTCH) gene expression. It is also thought to play a role during embryogenesis. The encoded protein is associated with several phenotypes- Greig cephalopolysyndactyly syndrome, Pallister-Hall syndrome, preaxial polydactyly type IV, postaxial polydactyly types A1 and B. [provided by RefSeq, Jul 2008]

Chr: Chromosome

***Supporting Information File 1*. Elegibility criteria for inclusion/exclusion from the study research and genetic data recorded for each patient**

Elegibility criteria	Genetic factors
Complete orthodontic treatment	Chromosome X-Y, 1,2,3,4,5,6,8,10,12,14,15,18,19
Complete root formation	
No previous history of dental trauma	
No systemic pathologies altering hard tissue biology	
No root canal treatment therapy on measured teeth	
No orthodontic retreatment	Candidate SNPs previously studied within EARR [rs1800587, rs1143634, rs419598, rs315952, rs11730582, rs11573856, rs2073618, rs731236, rs1718119,rs2230912, rs7237982, rs8086340, rs17069845, rs1805034, rs12970081, rs17069898, rs4426449]
No impacted canines proximate to incisors	
Available lateral and panoramic pre and post-treatment radiographs	
Genotyping data needed to be available	

***Supporting Information File 2*. Diagnostic, clinical and radiographic variables recorded for each patient**

Diagnostic data recorded	Clinical data recorded	Radiographic data recorded
Age [years]	Treatment time [months]	Teeth with the most EARR [12,11,21,22]
Sex [female, male]	Treatment type [fixed appliance, aligners]	Mean EARR (±SD) [mm]
Ethnic origin [West European, East European, Latin American or North Africa]	Treatment [extraction, non-extraction]	Malgrem score [0,1,2,3,4]
Dental Malocclusion [I,II/1, II/2, III]	Bracket slot type [0.018, 0.022]	Crown/root [ratio]
Hypoplasia [yes, no]	Treatment phases [1,2]	Apex morphology [curved, rounded, pointed, square]
Microdontia [yes, no]	Distalizer appliance [yes, no]	Crown lenght 1.2, 1.1 (T0, T1)
Asthma medication [yes, no]	Maxillary expansion [yes, no]	Root lenght 1.2, 1.1 (T0, T1)
Onychophagia [yes, no]	Dental expansion [yes, no]	Total lenght 1.2, 1.1 (T0, T1)
Oral breathing [yes, no]	Incorrect archwire sequencee [yes, no]	
Lingual piercing [yes, no]	Bracket rebonding [yes, no]	Vertical movement of the apex [mm]
Smoker [yes, no]	Intraoral class Elastics [yes, no]	Sagital movement of the apex [mm]
	Intraoral anterior Elastics [yes, no]	Incisor proinclination angle [º]
	Inset movement [yes, no]	Skeletal Malocclusion [I,II, III]
	Offset movement [yes, no]	
	Vertical forces applied [intrusion, extrusion, none]	
	Torque applied [yes, no]	
	ABO Discrepancy Index[+] [score]	

[+]**Discrepancy Index ABO**(from Cangialosi TJ, et al. The ABO discrepancy index: a measure of case complexity. Am J Orthod Dentofacial Orthop. 2004;125:270-278) ;

***Supporting Information File 3*. Quality control steps performed in the genotyped sample**

GWAS Quality control
1. Stratification: we check possible sample stratification perdorming a graphical analysis based on the "eigenstrat" method, which is based on principal component analysis [1]
2. Genotype missing control: the call rate of samples and SNPs analysed was higher than 0.90
3. Minor allele frequency (MAF): We checked out the MAF in all the SNPs to ensure that enough number of the rare genotypes was present in the dataset. We only included polymorphisms with MAF≥1%
4. Cryptic relatedness: We assumed that there wasn´t cryptic relatdness between study participants. Cryptic relatedness refers to refers to the idea that some subjects of a case-control study might in fact be close relatives, thus their genotypes being not independent from the population frequencies.

[1] Patterson N, Price AL, Reich D: Population structure and eigenanalysis. PLoS Genet 2006, 2:e190.

9. DISCUSIÓN

Los resultados globales del presente trabajo de tesis doctoral, abordados a través de cada uno de los tres capítulos, permiten extraer conclusiones definitivas en respuesta a una hipótesis global en torno a la aEARR. Tal es así que a partir de los resultados descritos en el Capítulo 1, se evidencia con una síntesis crítica, que en la literatura científica hasta la fecha no existen criterios unificados para el diagnóstico de la EARR, existiendo discrepancias significativas tanto en la técnica radiográfica utilizada, el tamaño de la muestra, el diente medido, el método de medición y la interpretación de los resultados obtenidos.

En el análisis crítico y la reflexión realizada [*Paper #1*], se observó una tendencia actual creciente hacia el uso de la **radiografía panorámica** para el estudio de la EARR, reemplazando el uso predominante previo de las radiografías periapicales en otros estudios clásicos, ya que estas últimas a menudo faltan en los registros ortodóncicos estándar. En los estudios incluidos, el más reciente que emplea radiografía periapical data de 2018 (76), mientras que entre 2018 y 2023 existen seis estudios que utilizan radiografía panorámica (24-26, 77-79). Esta tendencia, coincide con el protocolo empleado en el Máster de Ortodoncia de la Universidad Complutense de Madrid, donde el diagnóstico se realiza, junto con otras pruebas, con radiografías panorámicas y telerradiografías al inicio y al final del tratamiento de los pacientes. En consecuencia, los estudios llevados a cabo en los capítulos 2 y 3 emplean estas radiografías panorámicas como método diagnóstico de la EARR.

En cuanto al **diente seleccionado para la medición de la EARR**, algunos investigadores se enfocan en un incisivo superior específico (26), mientras que otros analizan 24 dientes por paciente (78). Esta variabilidad en la unidad y tamaño de muestral puede afectar la representatividad de los resultados, dado que la prevalencia de reabsorción radicular puede variar considerablemente al comparar 24 dientes de un mismo paciente con un solo diente. La selección del diente introduce factores de confusión adicionales y posibles sesgos de interpretación, ya que los efectos de la aplicación de fuerza sobre raíces de diferentes tamaños, e incluso de dientes unirradiculares y multirradiculares, pueden no ser directamente comparables (32). Para reducir estas discrepancias y lograr mayor coherencia, en la revisión del Capítulo 1 se incluyeron únicamente estudios centrados en los incisivos maxilares, los cuales presentan una mayor susceptibilidad a la reabsorción radicular (2, 35, 39). De esta forma, en el Capítulo 2, las mediciones se realizaron tanto en el incisivo central superior (1.1) como en el lateral (1.2) de cada paciente, con el objetivo de comparar la morfología entre ambos dientes y evaluar las diferencias entre distintos grupos étnicos. Y en los dos estudios del Capítulo 3 se seleccionó, entre los cuatro incisivos superiores, el diente con mejor visibilidad y mayor reabsorción, y las mediciones se realizaron exclusivamente sobre este único incisivo.

Asimismo, la utilización de métodos de medición heterogéneos en distintos estudios constituye una fuente significativa de sesgo en la interpretación final o global de los resultados útiles de la literatura, siendo particularmente relevante la elección de la **estructura de referencia empleada para corregir la magnificación de las radiografías**. Algunos autores optaron por utilizar una esfera metálica cementada al diente como referencia estable (76), mientras que otros recurrieron a la dimensión mesio-distal del molar (25). El 62% de los estudios analizados empleó la corona dental como estructura fija y la línea amelocementaria como punto de delimitación entre la corona y la raíz, siendo el método más prevalente utilizado el de Linge and Linge modificado por Brezniak (22). De manera similar, en los estudios desarrollados en los Capítulos 2 y 3 de esta investigación, se adoptó esta metodología, no obstante, durante el proceso de medición se presentaron algunas dificultades en la identificación precisa de esta estructura, lo que sugiere la necesidad de investigaciones adicionales orientadas a establecer una metodología más precisa y fiable para la evaluación de la EARR. Por ello, y con el propósito de mitigar los posibles errores derivados de la identificación de la línea amelocementaria, en los estudios realizados en el Capítulo 2 y en el segundo estudio del Capítulo 3, se optó por complementar las mediciones con el método visual de Malgrem (29), a fin de corroborar y reforzar la precisión de los resultados obtenidos.

La utilidad de realizar mediciones para evaluar la reabsorción radicular apical externa es cuestionable en ausencia de un consenso claro respecto **al umbral en el que se considera significativa la reabsorción**. Esta falta de acuerdo compromete la fiabilidad y comparabilidad de los resultados entre estudios, ya que diferentes autores establecen criterios variables para el inicio de la EARR. Algunos consideran un umbral de 0.1 mm (24, 25), mientras que otros sugieren 1.20 mm (80), 1.46 mm (75), o 2 mm (81). Para abordar esta variabilidad en los estudios, en el Capítulo 2 se estableció un criterio de EARR agresiva a partir de los 4 mm de reabsorción radicular. Con el objetivo de evitar cualquier ambigüedad en la clasificación de la severidad, se dividió la muestra en los grados Malgrem 0 (sin EARR) y Malgrem 3 y 4 (reabsorción severa/agresiva), excluyendo los grados 2 y 3 de Malgrem para garantizar una diferenciación clara y evitar potenciales sesgos en la interpretación de los resultados. Esta selección permite definir grupos de comparación bien delineados y facilita una interpretación robusta de la gravedad de la reabsorción. En el Capítulo 3, se siguió un enfoque similar para reforzar la precisión en la categorización de los grados de aEARR. En el primer estudio, se dividió la muestra en dos grupos: aquellos con reabsorciones menores a 5 mm y aquellos con reabsorciones superiores a 5 mm, proporcionando así una delimitación nítida entre pacientes con y sin reabsorción significativa. El segundo estudio, se llevó a cabo clasificando la muestra de acuerdo con los grados de severidad de Malgrem; los grados 0, 1, 2 y 3 se consideraron como grupo control, mientras que los de grado 4 se clasificaron como el grupo afectado por EARR agresiva. Al seleccionar el grupo afectado de aEARR exclusivamente un grado de reabsorción considerado agresivos en todos los estudios de esta investigación, se logra una diferenciación más clara entre los grupos de comparación, minimizando la posibilidad de confusión en la clasificación de la severidad de la reabsorción, lo que permite obtener resultados más precisos y robustos. Además, este criterio de selección contribuye a optimizar la capacidad del análisis para detectar asociaciones significativas, al reducir la variabilidad en los datos y mejorar la consistencia en la interpretación de los resultados. Este enfoque metodológico no solo permite una definición clara y sólida de los grupos de estudio, sino que también mejora la relevancia clínica y la aplicabilidad de los resultados al establecer un marco uniforme que facilita la comparación y análisis entre estudios, contribuyendo a una base más sólida para futuras investigaciones en el ámbito de la EARR.

Actualmente, parece plausible que la **inteligencia artificial** (AI) pueda proporcionar un método más estandarizado y criterios más homogéneos para un diagnóstico preciso de esta patología, y mientras que se ha demostrado recientemente la viabilidad de medir la reabsorción radicular con ayuda de la AI en CBCT (82), aún no se ha materializado en el ámbito de las radiografías panorámicas y periapicales.

No obstante, incluso con un método de medición de precisión impecable, las inconsistencias y la falta de fiabilidad en la literatura científica actual subrayan la importancia de establecer un consenso sobre el método diagnóstico, las técnicas de medición y los criterios de interpretación para la reabsorción radicular apical externa. La variabilidad en las metodologías de los distintos estudios contribuye a las discrepancias en las tasas de prevalencia reportadas y en los resultados del tratamiento, y dificulta el **establecimiento de protocolos estandarizados para la práctica clínica**. Por lo tanto, existe una necesidad urgente de esfuerzos colaborativos entre investigadores, clínicos y organizaciones profesionales para desarrollar guías universalmente aceptadas que abarquen la selección de modalidades de imagen, la estandarización de técnicas de medición y el establecimiento de criterios claros para la interpretación de la gravedad de la EARR. Un consenso no solo mejorará la comparabilidad de los hallazgos de investigación, sino que también facilitará el diagnóstico, monitoreo y manejo preciso de la EARR en entornos ortodónticos y otros ámbitos odontológicos.

Las limitaciones mencionadas, contribuyen de manera decisiva a la validez y relevancia clínica de las evaluaciones de la EARR y su asociación con factores de riesgo predisponentes.

La **duración del tratamiento** ortodóntico es uno estos factores, siendo el más estudiado y consistentemente asociado con un aumento en la incidencia y severidad de la EARR (31). Diversos estudios han demostrado que el tiempo prolongado de aplicación de fuerzas sobre los dientes incrementa el riesgo de reabsorción radicular apical (15, 32-34), por esta razón se le ha prestado especial atención en los diferentes estudios de esta tesis. En el Capítulo 2, los resultados corroboraron esta relación, evidenciando una asociación significativa entre el tiempo de tratamiento y la gravedad de la EARR. Sin embargo, en el Capítulo 1, no fue posible establecer de manera concluyente si la duración del tratamiento influye en el desarrollo de la EARR o descartar dicha influencia, lo que refleja la heterogeneidad en los hallazgos disponibles. Por otro lado, en el Capítulo 3 [*Papers #3 y #4*], no se identificaron asociaciones significativas entre la duración del tratamiento y la EARR, lo que sugiere que otros factores, probablemente relacionados con las características individuales de cada paciente, pueden también desempeñar un papel determinante en el desarrollo de esta condición.

Otro factor que puede influir en la susceptibilidad individual a la EARR es la **etnia.** En el contexto del mundo globalizado actual, resulta fundamental comprender las variaciones étnicas y genéticas entre las diferentes poblaciones, un aspecto que se ha explorado en el Capítulo 2 de esta tesis. En la literatura, se ha identificado que la morfología radicular es un factor de riesgo significativo para el desarrollo de la EARR (83), y se ha demostrado que la etnicidad influye en la forma y la anatomía dentales (84). En nuestro estudio, no se encontraron diferencias étnicas en el incisivo central superior; sin embargo, se observaron diferencias en el incisivo lateral superior, lo que coincide con hallazgos previos que sugieren que éste puede estar más predispuesto a variaciones morfológicas (85). Además, los pacientes latinoamericanos presentaron una mayor prevalencia de morfología de ápice puntiagudo ($p = 0.010$) y una menor tasa de EARR de severidad leve/moderada que los pacientes del sur de Europa. Esta observación plantea la posibilidad de que la morfología de ápice puntiagudo podría actuar como un factor protector frente a la EARR, lo que subraya la necesidad de realizar más investigaciones para explorar esta relación potencial. Asimismo, es notable que no se encontraron diferencias significativas en las formas más agresivas de severidad de la EARR entre diferentes grupos étnicos. Esta falta de variabilidad sugiere que, si bien la EARR leve a moderada puede estar influenciada por factores étnicos y morfológicos, la progresión a formas más severas podría estar gobernada por factores no relacionados con la etnicidad, lo que merece una mayor investigación. En la *scoping review* realizada en el Capítulo 1, se observó que la mayoría de los artículos no especifican las poblaciones de estudio incluidas en sus análisis. Esta falta de información puede influir significativamente en la interpretación de los resultados, ya que las variaciones étnicas podrían desempeñar un papel clave en la susceptibilidad a la EARR. En contraste, en el GWAS del *Paper #3* del Capítulo 3 se incluyeron polimorfismos y frecuencias alélicas específicas para una sola etnia (caucásica). El software empleado en el análisis garantiza la homogeneidad de la muestra al identificar y descartar automáticamente las diferencias genéticas y étnicas en los pacientes que no pertenecen al mismo grupo biogeográfico definido. En cambio, en el GWAS del *Paper #4,* al aumentar la muestra y la heterogeneidad étnica, se determinó la realización de un análisis genético con inclusión de un modelo multinivel con estratos étnicos múltiples para obtener resultados concluyentes que tuvieran en cuenta las diferencias étnicas de los pacientes. Consideramos imprescindible que futuros estudios incluyan esta variable para mejorar la comprensión y generalización de los hallazgos

En relación con **otros factores de riesgo asociados al desarrollo de la aEARR**, los resultados obtenidos en los dos estudios de asociación del genoma completo (GWAS) presentan diferencias significativas. En el primer estudio, realizado con una muestra de 480 pacientes, no se identificaron factores clínicos ni relacionados con los hábitos del paciente, siendo la predisposición genética el único elemento asociado a la susceptibilidad a la aEARR. En contraste, el segundo estudio, que incluyó a 612 pacientes, permitió identificar tres factores relevantes: el recementado repetido de brackets, la anchura del slot del bracket y un hábito del paciente, la onicofagia.

Esta discrepancia puede explicarse tanto por la diferencia en el tamaño muestral, cómo por la variabilidad en las características de las muestras de ambos estudios. Mientras que el primer GWAS incluyó pacientes provenientes de diversas clínicas, algunas fuera de Madrid, el segundo se limitó a pacientes tratados exclusivamente en el Máster de Ortodoncia de la Universidad Complutense. Esta diferencia en el origen de las muestras podría haber introducido heterogeneidad en los resultados. Además, el registro de la historia médica fue realizado por distintos profesionales en cada caso, lo que podría influir en la precisión de los datos relacionados con variables como hábitos y procedimientos clínicos. Cabe destacar que el registro de estas variables requiere un enfoque riguroso y detallado, y la certeza absoluta de las respuestas proporcionadas por los pacientes no siempre puede garantizarse. Estos hallazgos subrayan la complejidad multifactorial de la aEARR y destacan la importancia de un enfoque individualizado en la planificación y manejo del tratamiento ortodóntico. Además, enfatizan la necesidad de un registro clínico minucioso y estandarizado de la historia médica, que permita identificar con precisión los factores predisponentes en cada paciente, así como la importancia de seguir investigando estos factores mediante metodologías homogéneas y muestras representativas.

En esta investigación, ser **hombre** se ha identificado como un factor asociado a una mayor predisposición para desarrollar aEARR, tanto en el análisis genético del primer GWAS como en el análisis de factores específicos del paciente en el segundo GWAS. En el primer GWAS, se encontraron dos variantes genéticas, rs151184635 de STAG2 y rs55839915 de RP1-30E17.2, potencialmente implicadas en el fenotipo extremo de aEARR. Estas variantes, localizadas en el cromosoma X, se observaron exclusivamente en hombres, lo cual podría explicarse por el hecho de que los hombres poseen y heredan un único cromosoma X. En mujeres (XX) el fenómeno de inactivación de este cromosoma (lionización) puede contrarrestar los efectos de estas variantes genéticas y por tanto de la susceptibilidad a aEARR. Este hallazgo refuerza la importancia de considerar las diferencias genéticas y cromosómicas relacionadas con el género al estudiar la predisposición a la aEARR y su posible impacto en el desarrollo de estrategias preventivas y terapéuticas personalizadas.

En cuanto a las **variantes genéticas asociadas al desarrollo de aEARR**, en esta investigación no se identificaron asociaciones estadísticamente significativas sólidas [$p< 1x10^{-6}$] con ninguna de las variantes genéticas previamente estudiadas en la literatura, esto podría estar relacionado con las diferencias en la definición del fenotipo de EARR utilizado en las investigaciones. Mientras que muchos estudios previos han analizado variantes genéticas asociadas a la EARR en sus formas leves o moderadas, este trabajo se centra específicamente en el fenotipo agresivo (aEARR). No obstante, las diferencias puedan atender muy particularmente al grado de exigencia estadística mantenido para descartar falsos positivos. El nivel umbral se ha mantenido en un valor de $p < 1x10^{-4,}$ mientras en la literatura se consideran valores estándar de significación estadística en valores umbrales de $p < 0.05$, factor determinante a la hora de descartar falsos positivos en las asociaciones con genes candidatos. Al restringir el análisis a este tipo de EARR más severa, el umbral de significación podría haber disminuido, debido a que las variantes genéticas que influyen en formas menos agresivas de EARR podrían no tener un efecto relevante o detectable en un fenotipo tan extremo. Además, el enfoque en un fenotipo altamente específico contribuye a reducir la heterogeneidad de los casos analizados, lo cual es un paso esencial hacia una caracterización más precisa de los factores genéticos implicados. Este hallazgo resalta la importancia de estandarizar las definiciones fenotípicas en futuros estudios para garantizar la comparabilidad y obtener resultados más consistentes.

Por otro lado, la comparación entre los hallazgos genéticos de ambos GWAS del capítulo 3 y los resultados obtenidos, destacan avances significativos. En particular, se observa una asociación estadística más robusta con la aEARR en el *Paper #4* ($p < 1 \times 10^{-6}$) en comparación con los niveles de significación alcanzados en el *Paper #3* anterior ($p < 1 \times 10^{-4}$). Este aumento en la significación estadística subraya la importancia de una mayor muestra poblacional, que incrementó la potencia del análisis y permitió detectar efectos genéticos más sutiles. Además, en el *Paper #4* se realizó en análisis genético con inclusión de un modelo multinivel con estratos étnicos múltiples, lo que permitió identificar asociaciones genéticas más específicas y robustas relacionadas con la predisposición a la aEARR en los diferentes grupos étnicos.

Todas las variantes genéticas identificadas en ambos estudios (*STAG2, RP1-30E17.2, y IRX2)* parecen estar asociadas con procesos biológicos relacionados con la inflamación, la remodelación ósea, la resorción tisular, procesos de desarrollo y proliferación celular. Esto se alinea con las características patológicas observadas en la aEARR, donde la resorción ósea excesiva y las respuestas inflamatorias son elementos centrales del fenotipo. Estos hallazgos refuerzan la necesidad de que en investigaciones futuras se adopten metodologías estandarizadas y enfoques centrados en cohortes bien caracterizadas con una definición consistente del fenotipo aEARR. Ampliar el tamaño de muestra, diferenciar los grupos étnicos, diferenciar debidamente el fenotipo de EARR y aumentar la potencia significativa, será crucial para validar estas asociaciones y dilucidar las vías moleculares subyacentes, facilitando así el desarrollo de estrategias personalizadas para el manejo del riesgo en el tratamiento ortodóncico. Asimismo, sería fundamental considerar los tres genes y sus respectivos SNPs identificados en este estudio *(IRX2, SMAD2 y GLI2)* como marcadores clave para futuros estudios y análisis de genotipado.

Dado lo expuesto, resulta fundamental establecer un **protocolo diagnóstico y terapéutico estandarizado para la aEARR.** Esto permitiría una mejor comparabilidad entre los estudios, facilitando el avance en la comprensión de esta patología desde una perspectiva más consistente y homogénea.

En el ámbito diagnóstico, aunque la tomografía computarizada de haz cónico (CBCT) ofrece imágenes de alta calidad, su nivel de radiación aún no ha alcanzado los estándares deseados para garantizar la seguridad del paciente en evaluaciones rutinarias. Por tanto, las radiografías panorámicas continúan siendo la herramienta más recomendada para el diagnóstico habitual de la EARR. Tal como se ha discutido, los incisivos superiores, especialmente los laterales, presentan mayor susceptibilidad a la reabsorción radicular. Por ello, proponemos que los análisis de EARR se centren en estos cuatro dientes, seleccionando el más visible y con mayor grado de reabsorción para el análisis. Respecto a su medición, es crucial adoptar un método que sea tanto fiable como reproducible en radiografías panorámicas. Aunque la inteligencia artificial podría ofrecer soluciones en el futuro, actualmente sería conveniente incorporar una estructura fija en la ortopantomografía, como una regleta de referencia, para minimizar las magnificaciones y garantizar la precisión de las mediciones. Esto resulta más efectivo que la línea amelocementaria, cuya visualización no siempre es clara. Además, se sugiere complementar las mediciones digitales con métodos visuales, como la clasificación de Malmgren (29), para garantizar la coherencia en los resultados y proporcionar una categorización robusta de la EARR.

Es igualmente necesario alcanzar un consenso sobre el umbral a partir del cual se considera significativa la EARR. Los umbrales bajos pueden dar lugar a inconsistencias y errores; por ello, recomendamos establecer como referencia un umbral de reabsorción a partir de 4 mm o el estado 4 de Malmgren (29), que corresponde a la reabsorción de más de un tercio de la raíz.

Asimismo, resulta fundamental que los clínicos e investigadores realicen un registro detallado de todos los factores clínicos y características individuales del paciente que puedan influir en la EARR. Entre estos factores, deben incluirse hábitos como la onicofagia, factores como el recementado repetido de brackets y la anchura del slot, así como aspectos relacionados con la etnia del paciente. Esto último es de especial relevancia, ya que, como se ha argumentado anteriormente, las diferencias étnicas pueden influir en la morfología dental y, por ende, en la susceptibilidad a la EARR.

Algunos protocolos existentes sugieren realizar una radiografía periapical a los seis meses del inicio del tratamiento ortodóntico para detectar signos tempranos de EARR, basándose en evidencia que indica que la severidad de la reabsorción radicular observada en ese período podría predecir la progresión de la patología al final del tratamiento (86, 87). Este enfoque es razonable, ya que permite una intervención oportuna para modificar el plan de tratamiento si es necesario, reduciendo el riesgo de daño adicional. Sin embargo, es fundamental realizar más investigaciones para determinar el momento óptimo de las evaluaciones radiológicas y lograr una mayor estandarización y consenso en su implementación para garantizar su eficacia y aplicabilidad en diferentes contextos clínicos.

En el ámbito terapéutico de la EARR, se han investigado tratamientos dirigidos a influir directamente en la vía RANKL mediante la administración de senolíticos orales que han demostrado ser efectivos para reducir la expresión de RANKL en células senescentes inducidas por estrés mecánico, disminuyendo significativamente la EARR (11). Asimismo, se han estudiado otros tratamientos como la aplicación de láser (88) y la administración de litio (89), aunque ninguno ha alcanzado todavía un nivel de efectividad clínica consistente. Por ello, es imprescindible continuar investigando para desarrollar terapias específicas que no solo frenen la progresión de la aEARR, sino que también promuevan la regeneración de los tejidos afectados, lo cual representaría un avance significativo hacia un manejo clínico integral de esta complicación ortodóncica.

De esta forma, un enfoque protocolizado permitiría optimizar las estrategias de tratamiento para minimizar el impacto de la aEARR, proporcionando pautas específicas sobre la duración del tratamiento ortodóncico, el manejo de fuerzas mecánicas, y la identificación de pacientes de riesgo mediante un análisis previo de sus factores genéticos, clínicos y morfológicos.

Por último, es crucial destacar el papel de los factores genéticos en la susceptibilidad individual a la aEARR. En este contexto, se vislumbra un futuro en el que la evaluación genética previa al inicio del tratamiento ortodóncico podría convertirse en una herramienta esencial para la práctica clínica. Realizar estudios genéticos que permitan identificar variantes asociadas a un mayor riesgo de desarrollar aEARR no solo ayudaría a prevenir complicaciones, sino que también posibilitaría la personalización de los planes de tratamiento, optimizando la seguridad y eficacia de los procedimientos ortodóncicos. Aunque actualmente estas pruebas genéticas son costosas y no están al alcance de la mayoría de las prácticas clínicas, se espera que, con el avance de la tecnología y la reducción de costos, estas evaluaciones sean cada vez más accesibles. Este enfoque es ya una realidad en otras áreas de la medicina, donde los estudios genéticos se emplean para identificar riesgos hereditarios y guiar la toma de decisiones terapéuticas. Integrar estas herramientas en la ortodoncia abriría nuevas posibilidades para implementar estrategias preventivas y tratamientos más seguros y personalizados, minimizando el riesgo de reabsorción radicular y mejorando significativamente los resultados a largo plazo para los pacientes. Este progreso subraya la importancia de continuar investigando en esta área y promoviendo la integración de la genética en la práctica ortodóncica de precisión.

10. CONCLUSIONES

Fundamentados en los resultados obtenidos a lo largo de los 3 capítulos desarrollados en el presente trabajo, se pueden extraer las siguientes conclusiones generales:

1. Existe una falta de consenso respecto a los métodos de diagnóstico y medición de la reabsorción radicular apical externa, lo que conduce a inconsistencias en los resultados en la literatura. La estandarización de los protocolos diagnósticos y el perfeccionamiento de las técnicas de medición son esenciales para mejorar la precisión en la atención ortodóncica. [Capítulo 1, *Paper* #1]
2. La etnia influye en la susceptibilidad a la reabsorción radicular apical externa (EARR), particularmente en la morfología apical, la proporción corona/raíz y la severidad de la EARR en el incisivo lateral superior. Los pacientes latinoamericanos muestran una mayor prevalencia de morfología apical puntiaguda y una menor incidencia de EARR leve/moderada en comparación con los pacientes del sur de Europa. Sin embargo, no se observaron diferencias étnicas significativas en las formas más agresivas (aEARR). Además, los pacientes del sur de Europa presentan una proporción corona/raíz significativamente mayor en comparación con los pacientes del este de Europa. Asimismo, la duración del tratamiento es factor un crítico asociado a la EARR severa/extrema. Son necesarios planes de tratamiento personalizados que consideren las variaciones étnicas en la morfología dental y la susceptibilidad a la reabsorción radicular, con el objetivo final de mejorar los resultados para el paciente y promover la equidad en la atención ortodóntica. [Capítulo 2, *Paper* #2]
3. Múltiples variantes genéticas putativas localizadas en los cromosomas X e Y están potencialmente implicadas en un fenotipo agresivo de reabsorción radicular apical externa (aEARR). Las variantes genéticas *STAG2* rs151184635 y *RP1-30E17.2* rs55839915 se encontraron asociadas con un mayor riesgo de padecer aEARR, particularmente en hombres. [Capítulo 3, *Paper* #3]
4. La variante genética *IRX2* rs1122449, localizada en el cromosoma 5, está asociada con un incremento en el riesgo de aEARR en un estudio multinivel con incorporación de orígenes étnicos diferenciados. Asimismo, se identificaron asociaciones significativas entre factores clínicos como el sexo masculino, el reposicionado de brackets y la onicofagia con un mayor riesgo de aEARR. [Capítulo 3, *Paper* #4]

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12. APÉNDICE I: ABREVIATURAS

“: inches (pulgadas).

A

aEARR: Aggressive External Apical Root Resorption (reabsorción radicular apical externa agresiva).
ARIAS: Apical Resorption Image Analysis System (sistema de análisis de imagen para la reabsorción apical).
ATP: Adenosin Triphosphate (adenosín trifosfato).

C

CBCT: Cone Beam Computed Tomography (tomografía computerizada de haz cónico).
CEJ: Cement-Enamel Junction (línea amelocementaria).

E

EARR: External Apical Root Resorption (reabsorción radicular apical externa).

I

AI: Artificial Inteligence (inteligencia artificial).
IFN: Interferón.
IL: Interleuquina.

M

mm: milímetros.
MMPs: Matrix Metalloproteinases (metaloproteinasas de la matriz).

N

NO: Nitrid Oxid (óxido nítrico).

O

OPG: Osteoprotegerin (osteoprotegerina).
OPN: Osteopontin (osteopontina).

P

PDL: Periodontal Ligament (ligamento periodontal).

R

RANKL: Receptor Activator for Nuclear Factor κB Ligand (Ligando del Activador del Receptor del Factor Nuclear κB).

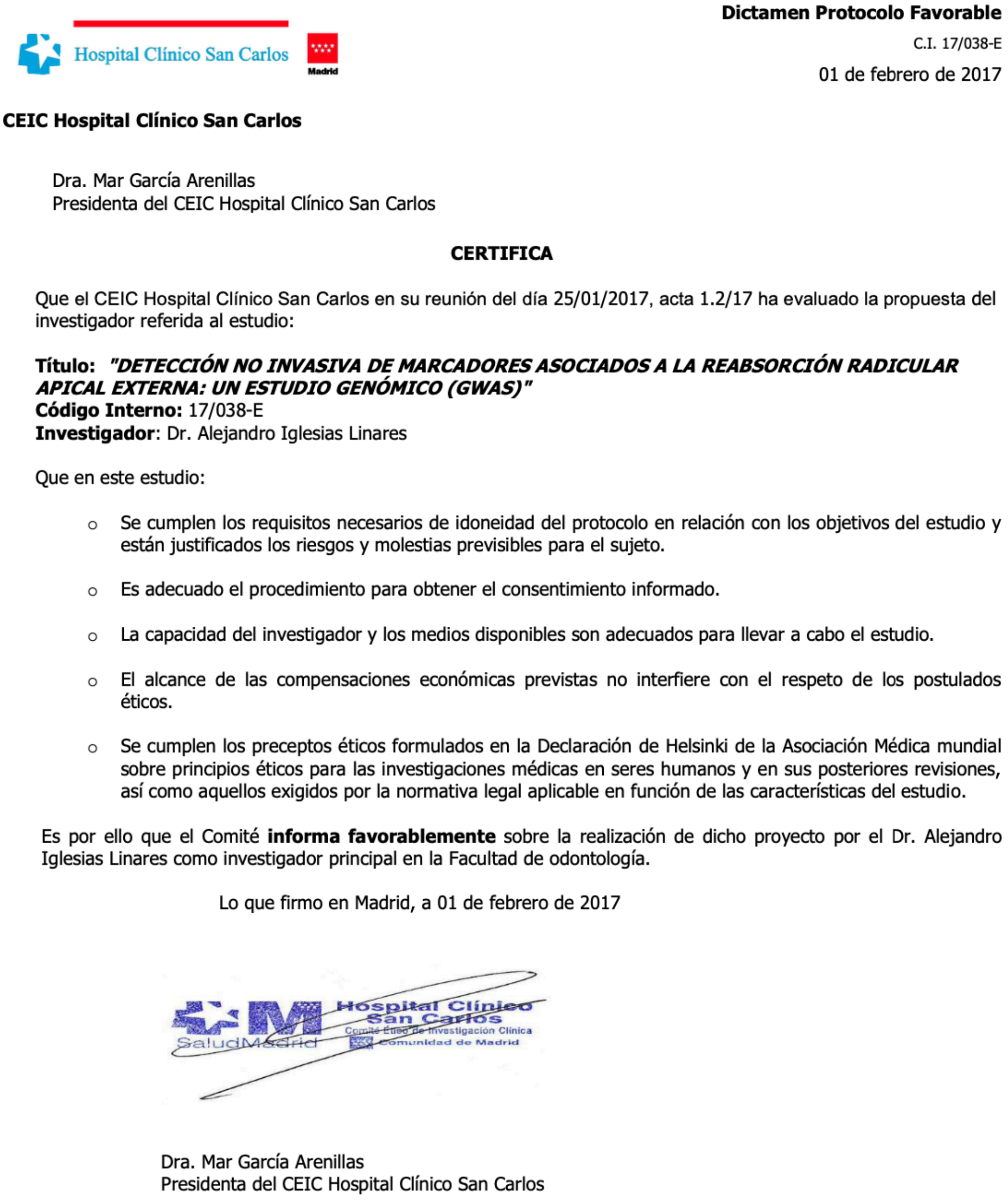
S

SNP: Single Nucleotid Polimorphism (polimorfismo de nucleótido único).

T

TNF: Tumor Necrosis Factor (factor de necrosis tumoral).

13. APÉNDICE II: APROBACIÓN DEL COMITÉ ÉTICO. URI 136-121124



Hospital Clínico San Carlos
Madrid

Dictamen Protocolo Favorable
C.I. 17/038-E
01 de febrero de 2017

CEIC Hospital Clínico San Carlos

Dra. Mar García Arenillas
Presidenta del CEIC Hospital Clínico San Carlos

CERTIFICA

Que el CEIC Hospital Clínico San Carlos en su reunión del día 25/01/2017, acta 1.2/17 ha evaluado la propuesta del investigador referida al estudio:

Título: ***"DETECCIÓN NO INVASIVA DE MARCADORES ASOCIADOS A LA REABSORCIÓN RADICULAR APICAL EXTERNA: UN ESTUDIO GENÓMICO (GWAS)"***
Código Interno: 17/038-E
Investigador: Dr. Alejandro Iglesias Linares

Que en este estudio:

- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Es adecuado el procedimiento para obtener el consentimiento informado.
- La capacidad del investigador y los medios disponibles son adecuados para llevar a cabo el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto de los postulados éticos.
- Se cumplen los preceptos éticos formulados en la Declaración de Helsinki de la Asociación Médica mundial sobre principios éticos para las investigaciones médicas en seres humanos y en sus posteriores revisiones, así como aquellos exigidos por la normativa legal aplicable en función de las características del estudio.

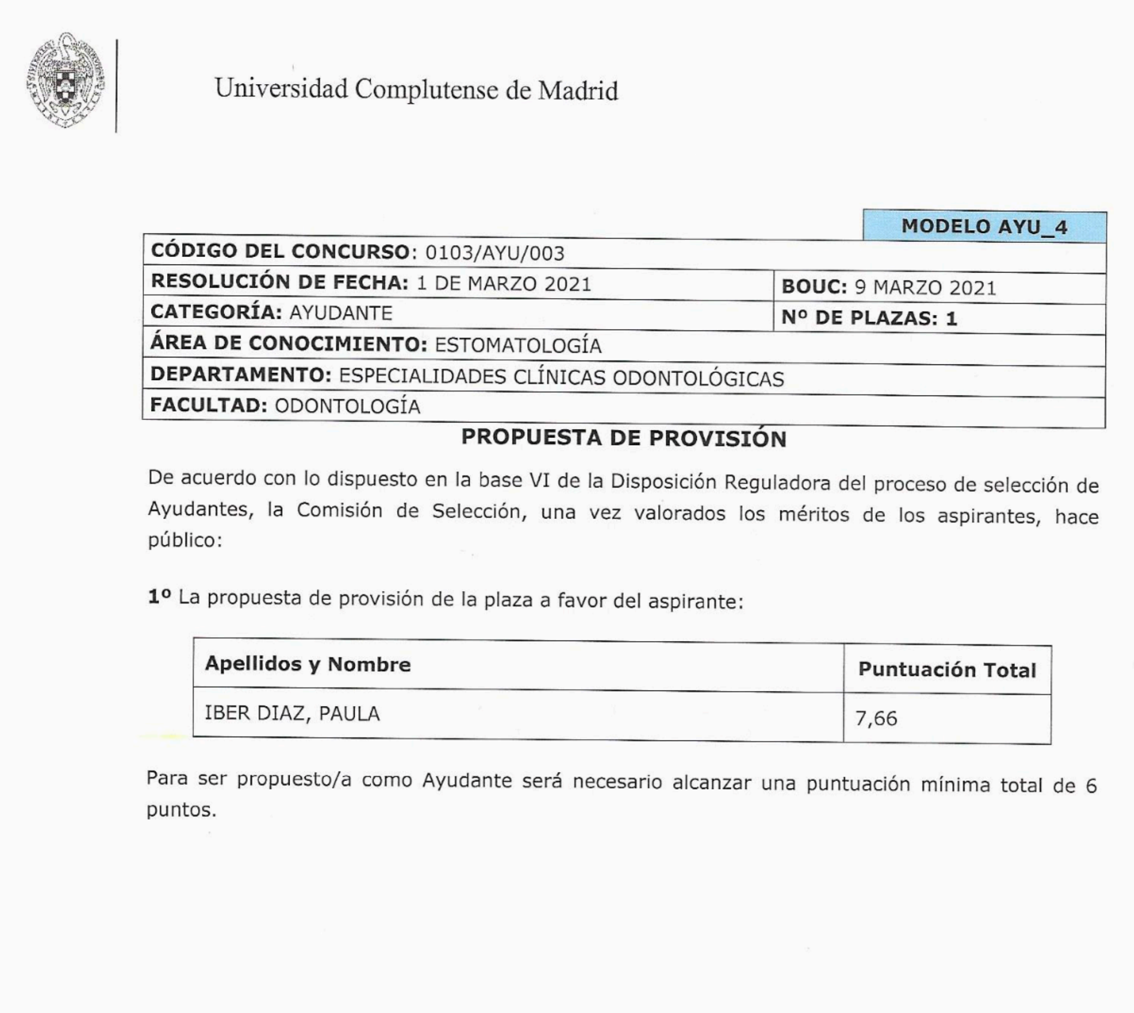
Es por ello que el Comité **informa favorablemente** sobre la realización de dicho proyecto por el Dr. Alejandro Iglesias Linares como investigador principal en la Facultad de odontología.

Lo que firmo en Madrid, a 01 de febrero de 2017

SaludMadrid
Hospital Clínico San Carlos
Comité Ético de Investigación Clínica
Comunidad de Madrid

Dra. Mar García Arenillas
Presidenta del CEIC Hospital Clínico San Carlos

14. APÉNDICE III: RESOLUCIÓN DEL CONTRATO DE AYUDANTE



Universidad Complutense de Madrid

MODELO AYU_4

CÓDIGO DEL CONCURSO: 0103/AYU/003

RESOLUCIÓN DE FECHA: 1 DE MARZO 2021 **BOUC:** 9 MARZO 2021

CATEGORÍA: AYUDANTE **Nº DE PLAZAS: 1**

ÁREA DE CONOCIMIENTO: ESTOMATOLOGÍA

DEPARTAMENTO: ESPECIALIDADES CLÍNICAS ODONTOLÓGICAS

FACULTAD: ODONTOLOGÍA

PROPUESTA DE PROVISIÓN

De acuerdo con lo dispuesto en la base VI de la Disposición Reguladora del proceso de selección de Ayudantes, la Comisión de Selección, una vez valorados los méritos de los aspirantes, hace público:

1º La propuesta de provisión de la plaza a favor del aspirante:

Apellidos y Nombre	**Puntuación Total**
IBER DIAZ, PAULA	7,66

Para ser propuesto/a como Ayudante será necesario alcanzar una puntuación mínima total de 6 puntos.