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Assessing the contribution of genes involved in monogenic bone disorders to the etiology of atypical femoral fractures

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Abstract

Background Recent studies suggested that genetic variants associated with monogenic bone disorders were involved in the pathogenesis of atypical femoral fractures (AFF). Here, we aim to identify rare genetic variants by whole exome sequencing in genes involved in monogenic rare skeletal diseases in 12 women with AFF and 4 controls without any fracture.

Results Out of 33 genetic variants identified in women with AFF, eleven (33.3%) were found in genes belonging to the Wnt pathway (*LRP5*, *LRP6*, *DAAM2*, *WNT1*, and *WNT3A*). One of them was rated as pathogenic (p.Pro582His in *DAAM2*), while all others were rated as variants of uncertain significance according to ClinVar and ACMG criteria.

Conclusions Osteoporosis, rare bone diseases, and AFFs may share the same genes, thus making it even more difficult to identify unique risk factors.

Keywords Atypical femoral fracture, AFF, WES, Monogenic bone disorder

Background

Atypical femoral fractures (AFF) are low-impact fractures that differ from classic osteoporotic femoral fractures in that they occur in the subtrochanteric region or femoral diaphysis, usually in the setting of prolonged treatment with bisphosphonates (BP).While its physiopathology has not been completely clarified, genetic predisposition appears to be key given that the incidence of AFF is very low in the general population (5.9 per 10,000 person-years) [1], and specific patient populations appear to be at an increased risk of sustaining them including people with Asian ancestry and patients affected with specific monogenic bone disorders [2]. A number of studies have tried to identify the genes involved in the AFF risk suggesting that AFF occur in the setting of a complex and heterogeneous genetic background where each affected individual could have their own genetic burden [3, 4]. In

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a recent study by Zhou et al. (2023) [5], the authors suggested that genetic variants associated with monogenic bone disorders might play a role in the pathogenesis of AFF. In their study, 37 candidate genes involved in monogenic bone disorders were analyzed from whole-exome sequencing (WES) data in 60 AFF patients, with 95% having received bisphosphonates. Fifteen (25%) of the 60 AFF patients had clinical features of a monogenic bone disorder. In total, nine patients in their AFF cohort (15%) had a (likely) pathogenic variant, eight of whom fulfilled clinical criteria suggestive of monogenic bone disorders.

In a prior study performed by our group [6], we selected genetic variants in genes shared by at least two AFF patients and absent in controls. Hence, those variants only present in one AFF patient were removed from the analysis.

In view of the recent studies [3, 4] where each individual could have their own genetic background and furthermore, genes involved in monogenic bone disorders can play an important role, we decided to contribute with new data on 12 women with AFF and 4 controls without any fracture, all of whom had received bisphosphonates for over 5 years due to a diagnosis of osteoporosis. In the present study, we aimed to recover all rare genetic variants present in our AFF patients and controls from a list of 37 candidate genes proposed by Zhou et al. [5] and other additional genes involved in rare monogenic diseases with bone impairment.

Methods

Participants were previously described in a work published by our group [6]. Briefly, twelve unrelated postmenopausal women with AFF (mean age±SD of 74.5±6.1) and four postmenopausal women without any fracture (controls) (mean age±SD of 79±7.2) were recruited in Hospital del Mar (Barcelona, Spain) and Hospital Universitario de La Princesa (Madrid, Spain). All of them received bisphosphonate (BP) treatment for >5 years due to a diagnosis of osteoporosis. No patient had hypophosphatemia or suspicion of a monogenic disease. Half of the AFF patients had received corticosteroid therapy for more than one year due to polymyositis, rheumatoid arthritis, asthma or chronic bronchitis. None of the controls had receive glucocorticoids.

Whole exome sequencing (WES) was performed at the CNAG facilities (Barcelona, Spain). Capture was performed using Agilent Human All Exon 50 Mb v5 and samples were sequenced at a coverage of 140x on a HiSeq 2000 sequencer. Pipeline of the WES is detailed in Garcia-Giralt et al. (2022) [6].

We filtered for rare variants (MAF<0.005) with CADD>20 and the resulting variants were overlapped with our list of candidate genes (Supplemental Table 1).

Results and discussion

A total of 41 genetic variants were detected in genes belonging to the list of candidate genes (Table 1). Only one of the variants identified was rated as pathogenic according to ClinVar or ACMG guidelines (p.Pro582His in *DAAM2* [7]), while all others were rated as variants of uncertain significance (VUS) according to ACMG criteria.

Interestingly, variants in *COL1A1* and *COL1A2* were detected in control individuals (with osteoporosis and long-term BP treatment, but without AFF), suggesting their putative involvement in the underlying osteoporosis phenotype. The same could be proposed for the *FKBP10* and *TNXB* genes, which were found mutated in controls and AFF patients. Zhou et al. [5] found likely pathogenic variants and VUS in *COL1A1* and *COL1A2* genes related to a diagnosis of osteogenesis imperfecta or monogenic osteoporosis in 5 AFF patients, which could suggest a role of these genes in both bone tissue pathology and AFF.

Out of 33 genetic variants identified in women with AFF, eleven (33.3%) were found in genes belonging to the Wnt pathway (*LRP5*, *LRP6*, *DAAM2*, *WNT1*, and *WNT3A*). Moreover, the AFF11 woman was homozygous for a mutation in *DAAM2*. It is difficult to discern whether these genes play a role in the pathophysiology of AFF in addition to their known role in low bone mass. Similarly, *LRP5* was also found mutated in 2 AFF patients with a diagnosis of monogenic osteoporosis in the Zhou et al. study [5] suggesting this putative dual role. On the other hand, *DAAM2* gene was not assessed in the AFF patients from that study and we cannot know its involvement in bone phenotypes.

Subject AFF1 did not carry any mutations from our list of candidate genes while all other AFF patients were carriers of variants in more than one gene. For example, subject AFF11 accumulated up to 9 rare genetic variants, one of them in homozygosity (*DAAM2*). Unfortunately, there is no information about this latter variant in the ClinVar database. Interestingly, *Daam2* KO mice showed a marked reduction in bone strength, despite minimal changes in bone morphology and mineral content, indicating an abnormal bone composition and structure explained in part by cortical impairment [8].

While none of our patients could be confidently diagnosed of any monogenic bone disorder, like most of the Dutch AFF cohort [5], all shared the common feature of severe postmenopausal osteoporosis that required long-term BP treatment and, in some cases, also denosumab. Noteworthy, 50% of the women with AFF in our cohort and none of the controls had been on long-term glucocorticoid treatment which is a recognized risk factor for AFF [9]. When comparing both cohorts according to the gene candidate list proposed by Zhou et al. [5] (see

Table 1 Genetic variants found in AFF patients and controls detected by WES

ID Patient	Gene	Genetic variant	CADD Score	ALT frequency	CLINVAR	ACMG classification
AFF2	*FAM20C*	p.Ser410Thr	25.8	0.003385	Benign/Likely benign	VUS
AFF2	*LMNA*	p.Arg401Cys	34	0.00003295	VUS	VUS
AFF2	*TNXB*	p.Val1831Met	25.1	0.00005775	VUS	VUS
AFF2	*TNXB*	p.Asp677Gly	23.1	0.002365	VUS/likely benign	VUS
AFF3	*LRP6*	p.Ser1385Cys; p.Ser1476Cys; p.Ser1521Cys	26.2	0.000008236	No inf	VUS
AFF3	*WNT3A*	p.Tyr260Phe	23.4	No inf	No inf	VUS
AFF4	*DAAM2*	p.Arg990Leu	27.1	0.001183	No inf	VUS
AFF4	*WNT1*	p.Gly259_Gly262dup	21.9	0.000002787	No inf	VUS
AFF4	*ZMPSTE24*	p.Ser27Phe	23.4	0.00002471	No inf	VUS
AFF5	*LRP5*	p.Arg258Cys	34	0.00001648	VUS	VUS
AFF5	*PTH1R*	p.Tyr221Cys	26.2	0.000008238	VUS	VUS
AFF6	*LRP5*	p.Ser1482Leu	35.35	0.00003	VUS	VUS
AFF6	*LRP5*	p.Arg1036Gln	24.3	0.002487	VUS/likely benign	VUS
AFF6	*PLEKHM1*	p.Arg103His; p.Arg1047His; p.Arg958His	34	0.0004942	No inf	VUS
AFF6	*SLC34A3*	p.Lys298del	No inf	No inf	No inf	VUS
AFF7	*SLC34A3*	p.Arg468Gln	29.2	0.00004129	No inf	VUS
AFF8	*LRP5*	p.Pro1504Leu	34.34	0.0004	VUS/likely benign	VUS
AFF8	*MMP2*	p.Glu166Lys	24	0.001688	Benign/Likely benign	VUS
AFF9	*AHNAK*	p.Lys1438Asn	22.9	0.0002965	No inf	VUS
AFF9	*DAAM2*	p.Pro582His	22.8	0.001	Pathogenic	likely pathogenic
AFF10	*DAAM2*	p.Lys776Thr	28,2	0.0008113	No inf	VUS
AFF10	*ANKH*	p.Arg36Trp	34	0.00001647	No inf	VUS
AFF11	*AHNAK*	p.Glu5dup	No inf	No inf	No inf	VUS
AFF11	*ATP6V0A2*	p.Arg141Leu	25.6	0.0002883	VUS/likely benign	VUS
AFF11	*BMP1*	p.Arg371His	33	0.003706	Benign/Likely benign	VUS
AFF11	*CUL7*	p.Arg183Gln	24.4	0.00005765	VUS	VUS
AFF11	*DAAM2*	p.Pro555Leu/p.Pro555Leu*	24.7	0.0008105	No inf	VUS
AFF11	*FKBP10*	p.Ile436Thr	28.6	0.002	VUS/likely benign	VUS
AFF11	*PTH1R*	p.Pro581Arg	25.7	0.00008237	VUS	VUS
AFF11	*SLC34A3*	p.Arg568Cys	24.1	0.00003038	VUS	VUS
AFF11	*TNXB*	p.Arg2889Trp	21.9	0.0001573	VUS	VUS
AFF12	*CUL7*	p.Gly872Ser	34	0.001392	VUS/likely benign	VUS
AFF12	*PYCR1*	c.-112 C>T	34	0.0005564	No inf	VUS
Control2	*COL1A1*	p.Pro417Ser	22.6	0.00008238	VUS/likely benign	VUS
Control2	*IFIH1*	c.1641+1G>C	25.9	0.006483	VUS/Benign	VUS
Control2	*NOTCH2*	p.Arg1048His	22.8	0.00005765	VUS	VUS
Control2	*XYLT2*	p.Ala614Val	21.7	0.0006342	VUS	VUS
Control3	*ANO5*	p.Glu185Gln	26.3	0.0002471	VUS	VUS
Control3	*COL1A2*	p.Arg906His	27.8	0.0001235	VUS	VUS
Control3	*TNXB*	p.Val1482Met	25.9	0.001581	Benign/Likely benign	VUS
Control4	*FKBP10*	p.Gly286Arg	30	0.000008236	VUS	VUS

Abbreviations: ALT, alternative allele; ACMG, American College of Medical Genetics and Genomics; VUS, Variant of Uncertain Significance; AFF, atypical femoral fracture. *AFF11 is homozygous for the variant p.Pro555Leu

supplemental Table 1) and considering all AFF patients with or without a clinical suspicion of monogenic bone disorders, a similar number of carriers of variants in Mendelian bone disease genes was detected (46% in Zhou et al. vs. 50% in this cohort).

Conclusion

Encompassing all of our findings we conclude that in our cohort we did not detect a major gene involved in AFF pathophysiology. We speculate that AFF development is probably the result of the sum of genetic variants together with other structural, physiological and environmental factors. If osteoporosis, rare bone diseases, and AFFs share the same genes, identifying unique risk factors could be even more challenging.

These results warrant further studies of genes related to monogenic bone disorders in the setting of severe osteoporosis, in addition to their potential role in AFF pathogenesis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40246-024-00652-2.

Supplementary Material 1

Supplementary Material 2

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Author contributions
Conceptualization: N.G.G., D.G., S.B. and R.R.; Patient recruiting and phenotyping: D.O., S.C. and X.N.; Methodology: N.G.G., D.G., S.B., and R.R.; Validation: N.G.G. and R.R.; Formal Analysis: N.G.G. and R.R.; Investigation: N.G.G., D.G., S.B., and R.R.; Data Curation: all authors; Writing—Original Draft Preparation, N.G.G. and D.O.; Writing—Review & Editing: All authors; Supervision: S.B., D.G., X.N. and R.R.; Project Administration: N.G.G.; Funding Acquisition: N.G.G., X.N., S.C., S.B. and D.G. All authors have reviewed and agreed to the published version of the manuscript.

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Data availability
The datasets generated and/or analysed during the current study are not publicly available due extensive genomic information but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Clinical Research Ethics Committee of Parc de Salut Mar (CEIC; 2017/7717/I).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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