Atypical Femoral Fractures Related to Bisphosphonate Treatment

Subjects: Medicine, Research & Experimental | Genetics & Heredity

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Atypical femoral fractures (AFF) are rare fragility fractures in the subtrocantheric or diaphysis femoral region associated with long-term bisphosphonate (BP) treatment. The etiology of AFF is still unclear even though a genetic basis is suggested.

atypical femoral fractures bisphosphonates

1. Introduction

Atypical femoral fractures (AFF) are a very rare type of bone fractures associated mainly with bisphosphonates (BP) and very rarely also with denosumab use [1][2][3]. Genetic factors have been suggested as a possible explanation for both the higher risk of AFF in Asian populations and the low proportion of BP users that develop AFF [4].

Many attempts have been made to identify these genetic factors that may predispose some BP users to sustain AFF. Among them, a few studies have revealed that genetic variants in genes implicated in the mevalonate pathway, which is targeted by BP, may affect bone mineral density, bone turnover, and predispose to AFF, in response to BP treatment [5][6][7][8]. However, a recent genome-wide association study (GWAS) and candidate gene study comparing 51 AFF cases to 324 BP-treated controls was unable to find evidence of common genetic variants for BP-associated AFF [9]. Hence, the authors proposed to perform GWAS with a larger sample size as well as whole-exome or whole-genome sequencing studies. This combination of studies would help to uncover the genetic background associated with BP-related AFF, which has a high genetic heterogeneity, sometimes associated with monogenic disorders [10][11] or otherwise with a polygenic etiology and large variability among individuals [12][13].

2. Variant Selection

In order to identify genes putatively involved in AFF, authors first removed all variants identified in the four control samples, and then selected those genes harboring rare genetic variants (ExAC and CSVS < 0.005) shared in at least two patients (**Figure 1**). Authors identified 100 rare variants in 85 genes that were shared by at least two patients. In addition, 483 genes presented a rare variant in at least 2 patients with AFF (same gene, different variant). In total, 1006 variants in 455 genes were identified.

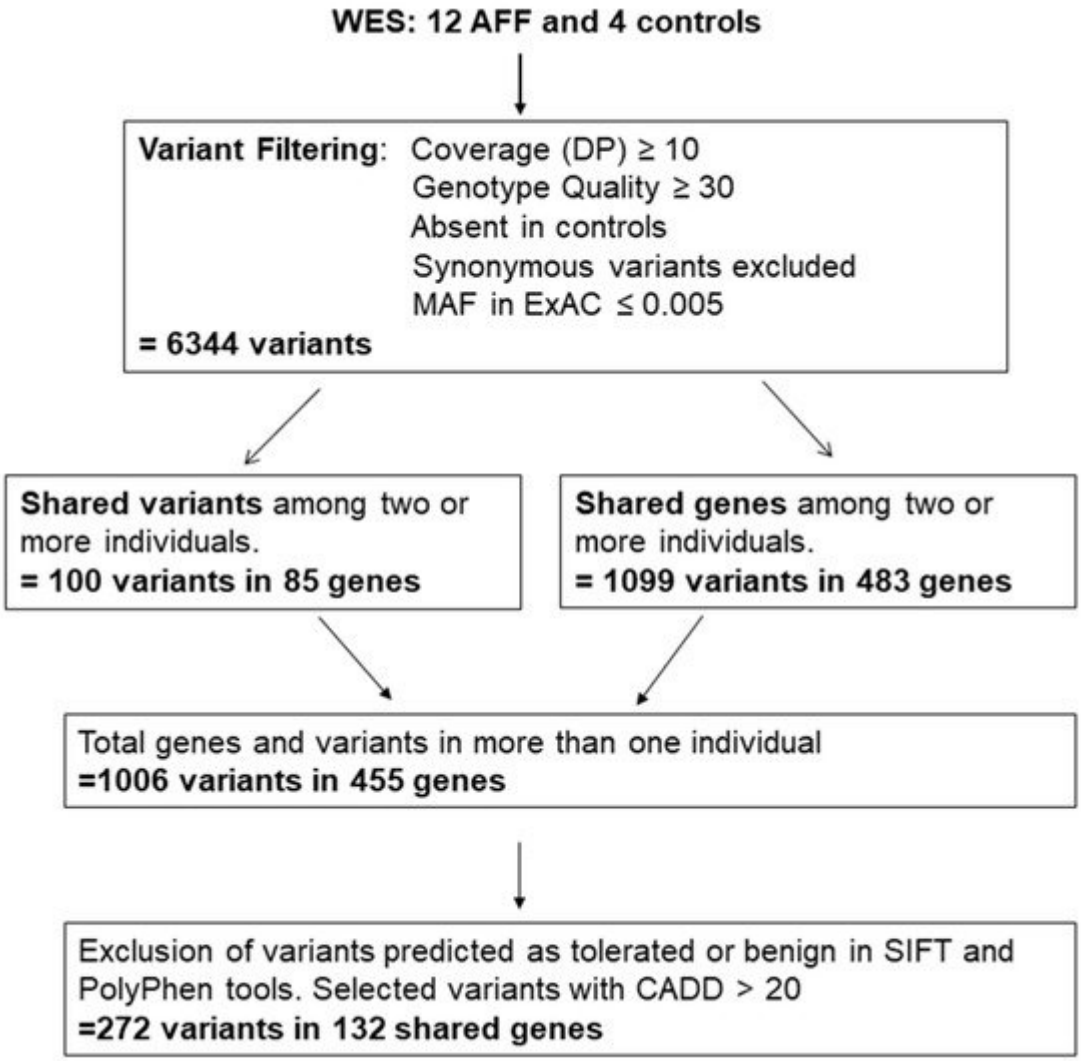


Figure 1. Pipeline of selected variants obtained by whole exome sequencing of 12 patients with BP-related AFF and 4 controls (individuals with long-term BP treatment without AFF). Only variants or genes mutated in at least two patients were considered for further analysis.

Variants were then prioritized based on functional prediction (excluding variants with CADD score < 20, and those considered tolerated or benign by SIFT or PolyPhen_humDiv, respectively). Considering only genes with at least two carriers of a rare variant, a total 272 variants in 132 genes remained (**Figure 1** and **Table 1**).

Table 1. Genes with at least two individuals carrying a rare variant; Variants were prioritized based on functional prediction (excluding variants with CADD score <20, and those considered tolerated or benign by SIFT or PolyPhen_humDiv, respectively).

Genes with Rare Variants in Two AFF Cases				Genes with Rare Variants in More Than two AFF Cases	
Two Different Variants		One Variant			
Gene Name	Gene Name	Gene Name	Gene Name	Gene Name	Number of Variants and (Carriers)
AASS	*DNAH10*	*PSD3*	*ACADL*	*C8orf46*	1 (3)

Genes with Rare Variants in Two AFF Cases				Genes with Rare Variants in More Than two AFF Cases	
Two Different Variants		One Variant			
Gene Name	Gene Name	Gene Name	Gene Name	Gene Name	Number of Variants and (Carriers)
ABCA10	*DNAH12*	*PTH1R*	*C1orf87*	*CHRNG*	3 (3)
ABCA4	*DNAH6*	*PYHIN1*	*CD1A*	*DAAM2*	3 (3, one homoz)
ABL2	*DYSF*	*R3HDML*	*CITED4*	*DNAH14*	4 (4)
ADAMTS12	*EFHB*	*RET*	*GBA*	*DNAH2*	3 (3)
ANAPC11	*EP400*	*RMDN1*	*IQSEC3*	*DNAH9*	3 (3)
ANK3	*ERCC5*	*RNF157*	*NSMAF*	*FSIP2*	3 (3)
ANKRD40	*FAT4*	*RNF34*	*PPP2R1B*	*HLA-DRB1*	2 (4)
ARHGEF18	*FBLN7*	*RTEL1*	*SERPINB2*	*HRASLS*	1 (3)
ARID1B	*FLJ00418*	*SCN9A*	*SPTBN1*	*IGFLR1*	2 (2, one homoz)
ASH1L	*GBP3*	*5-Sep*	*SYDE1*	*KRT10*	1 (5)
ATAD2	*GPX4*	*SH3BP2*	*TNFRSF25*	*LAMA1*	3 (3)
ATP10B	*HK3*	*SHROOM4*	*TRAPPC2L*	*LRP5*	4 (3)
BIN1	*HPS6*	*SIRT5*	*TRIM32*	*MRPS12*	1 (3)
C10orf54	*IGFN1*	*SLC26A9*		*NEB*	4 (4)
C12orf42	*IGSF10*	*SLC2A7*		*OBSCN*	5 (5)
C14orf159	*IGSF22*	*SLC34A3*		*TCOF1*	3 (4)
C17orf107	*KLHL33*	*SLC52A2*		*TNXB*	3 (3)
C6	*LLGL1*	*SPTBN5*		*TTN*	8 (8)
C9orf84	*MEX3D*	*SRCAP*		*UTRN*	3 (3)
CA9	*MKS1*	*TAF15*		*VEGFB*	1 (3)
CDC42BPG	*MMP20*	*TENM4*		*ZC3H3*	3 (3)
CERKL	*MSLNL*	*TJP3*			
CHAMP1	*NOD2*	*TMEM143*			
CLCN2	*NUP153*	*TNRC6B*			

Genes with Rare Variants in Two AFF Cases				Genes with Rare Variants in More Than two AFF Cases	
Two Different Variants		One Variant			
Gene Name	Gene Name	Gene Name	Gene Name	Gene Name	Number of Variants and (Carriers)
CRYBA1	*OPLAH*	*TOPORS*			
CTSE	*PACSIN2*	*TSFM*			
CUL7	*PARD6B*	*TTC14*			
CYYR1	*PCDHAC1*	*ZNF34*			
DAB2IP	*PDE4DIP*	*ZNF646*			
DAW1	*PISD*	*ZNF729*			
DHX34	*PLA2G4D*	*ZSCAN32*			

Function enrichment analysis using the BinGO and GeneMANIA app in Cytoscape yielded adjusted significant scores for dynein complex, contractile fiber, microtubule motor activity, ciliary transition zone, actin cytoskeleton organization and pyrophosphatase activity.

Afterwards, authors intersected this list with previously described genes involved in bone metabolism and/or AFF [13][14][15][16]. Twelve genes were identified and selected as candidate genes (**Table 2**) for further in silico analyses using the AFFNET tool. Half of the AFF patients were carriers of variants in one Wnt signaling gene: *DAAM2* (3 carriers, one each for p.(P555L) (homozygous), p.(P582H) and p.(R989L), and a fourth with a variant predicted as tolerated by SIFT (p.(K776T))) and *LRP5* (3 carriers, one each for p.(R258C) and p.(P1504L) and one carrying two variants (p.(R1036Q) and p.(S1482L)), suggesting a role of this pathway in AFF triggering.

Table 2. Genes involved in bone metabolism and/or AFF containing deleterious rare variants in at least two AFF patients of this study.

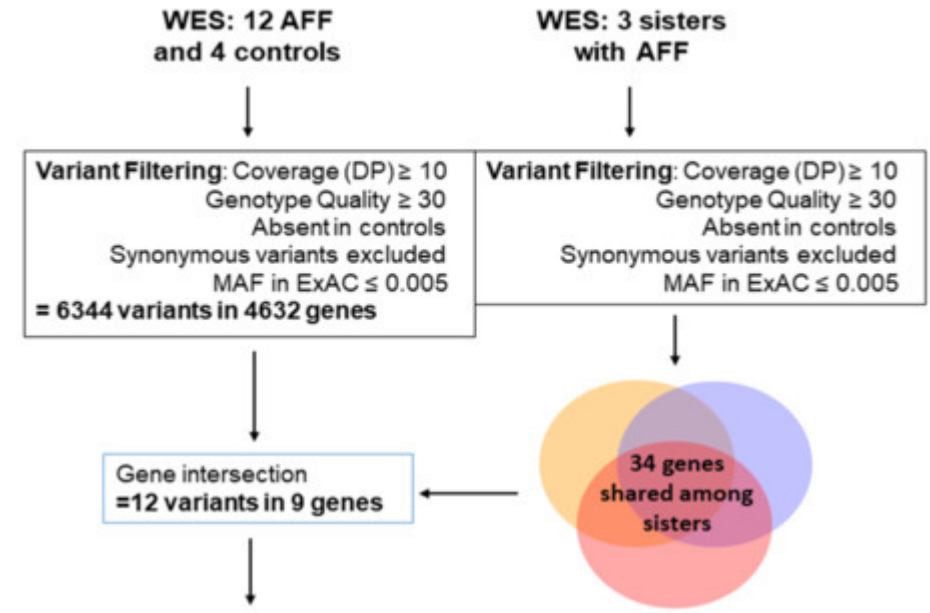
Gene ID	Number of Carriers	Function	Bone Association	Bibliography Source
CUL7	2	A core component of the 3 M complex required to regulate microtubule dynamics and genome integrity	Mutations in this gene produce the 3 m syndrome, which causes skeletal abnormalities	Genecards
DAAM2	3	Involved in the canonical Wnt signaling, a pathway critical for bone formation and repair	SNPs in this gene are associated with estimated bone mineral density (eBMD). *Daam2* knockout mouse showed decreased bone strength	Musculoskeletal Knowledge Portal, Morris et al., 2019 [17]

Gene ID	Number of Carriers	Function	Bone Association	Bibliography Source
DNAH10	2	Found in cilia and flagella; ATPase activity and microtubule motor activity	SNPs in this gene are associated with waist-hip ratio and eBMD.	Musculoskeletal Knowledge Portal
DNAH12	2	ATPase activity and microtubule motor activity	SNPs in this gene are associated with waist-hip ratio and eBMD	Musculoskeletal Knowledge Portal
LAMA1	3	A major component of the basal membrane which has been implicated in a wide variety of biological processes including cell adhesion, differentiation, migration, and signaling	Binding to cells via a high affinity receptor, laminin is thought to mediate the attachment, migration and organization of cells into tissues during embryonic development by interacting with other extracellular matrix components.	Genecards
LRP5	4	A co-receptor with Frizzled protein family members for transducing signals by Wnt proteins	It plays a key role in skeletal homeostasis and many bone density related diseases are caused by mutations in this gene	Genecards
MEX3D	2	RNA binding protein, may be involved in post-transcriptional regulatory mechanisms	Found mutated in three sisters with AFF	Roca-Ayats N, et al. 2018 [12]
PTH1R	2	A receptor for parathyroid hormone (PTH) and for parathyroid hormone-like hormone (PTHLH).	Involved in the Hedgehog and PTH signaling pathways in bone and cartilage development	Genecards
SLC34A3	2	Involved in the transporting phosphate into cells via sodium cotransport in the renal brush border membrane, and contributes to the maintenance of inorganic phosphate concentration in the kidney	Mutations in this gene are associated with hereditary hypophosphatemic rickets with hypercalciuria.	Genecards
SPTBN1	2	Spectrin is an actin crosslinking and molecular scaffold protein that links the plasma membrane to the actin cytoskeleton, and functions in the determination of cell shape, arrangement of	SNPs in this gene are associated with eBMD and total body BMD	Musculoskeletal Knowledge Portal

Gene ID	Number of Carriers	Function	Bone Association	Bibliography Source
		transmembrane proteins, and organization of organelles		
TNRC6B	2	Involved in cellular senescence, innate or adaptive immune system, Wnt signaling, and calcium modulating pathways	SNPs in this gene are mainly associated with lean mass. One SNP was also associated with lower lumbar spine BMD and increased risk of fractures	Karasik D, et al. 2019 [18]
TNXB	3	A member of the tenascin family of extracellular matrix glycoproteins	Mutations in this gene are associated with the Ehlers-Danlos Syndrome	Genecards

Genecards: https://www.genecards.org/, accessed on 9 December 2021. Musculoskeletal Knowledge Portal (MSK portal): https://msk.hugeamp.org/, accessed on 9 December 2021.

In parallel, authors compared all genes carrying rare variants in this study with previous results obtained from a WES in three sisters with BP-related AFF [12]. A total of 9 genes were found overlapping both studies (**Figure 2**). Four of them carried damaging rare variants: *LURAP1L, MEX3D, POLI*, and *SYDE2*. These genes were also considered candidate genes for the network analysis.



Gene_Name	Annotation	HGVS.p	dbNSFP_CADD_phred	dbNSFP_ExAC_Adj_AF	dbNSFP_Polyphen2_HDIV_pred	dbNSFP_SIFT_pred
SYDE2	stop_gained	p.Arg1058*	44	0.00002486		
MEX3D	missense_variant	p.Thr465Lys	28.7		D	D
LURAP1L	missense_variant	p.Cys226Phe	25.9		D	D
HEPHL1	missense_variant	p.Arg432Trp	24.9	0.001612	D	T
HEPHL1	missense_variant	p.Glu1022Lys	24.4	0.004742	B	T
POLI	missense_variant	p.Met419Val	23.7	0.001613	D	D
SNAPC4	missense_variant	p.Val748Met	23.5	0.001495	P	T
MEX3D	missense_variant	p.Ala148Val	23.1	0.000024	P	D
NGEF	start_lost	p.Met1?	20.8	0.000031	B	D
SNAPC4	missense_variant	p.Arg651Cys	15.4	0.00007611	B	D
BRAT1	missense_variant	p.Arg777Gly	1.518	0.00006197	B	T
FN1	missense_variant	p.Asn1837Ser	0.003	0.000004	B	T

Figure 2. All mutated genes from the WES were intersected with genes also mutated in a previous study with 3 sisters who sustained AFF [12].

3. AFF Network Analysis with Candidate Genes

Interactions among identified genes were explored using the AFFNET tool. In order to simplify the network display, the shortest path interactions among bone-related genes (described in **Table 2**) were explored. Therefore, only direct interactions between candidate genes are displayed. Candidate genes were interconnected with each other, even though in some cases through other intermediate genes (**Figure 3**).

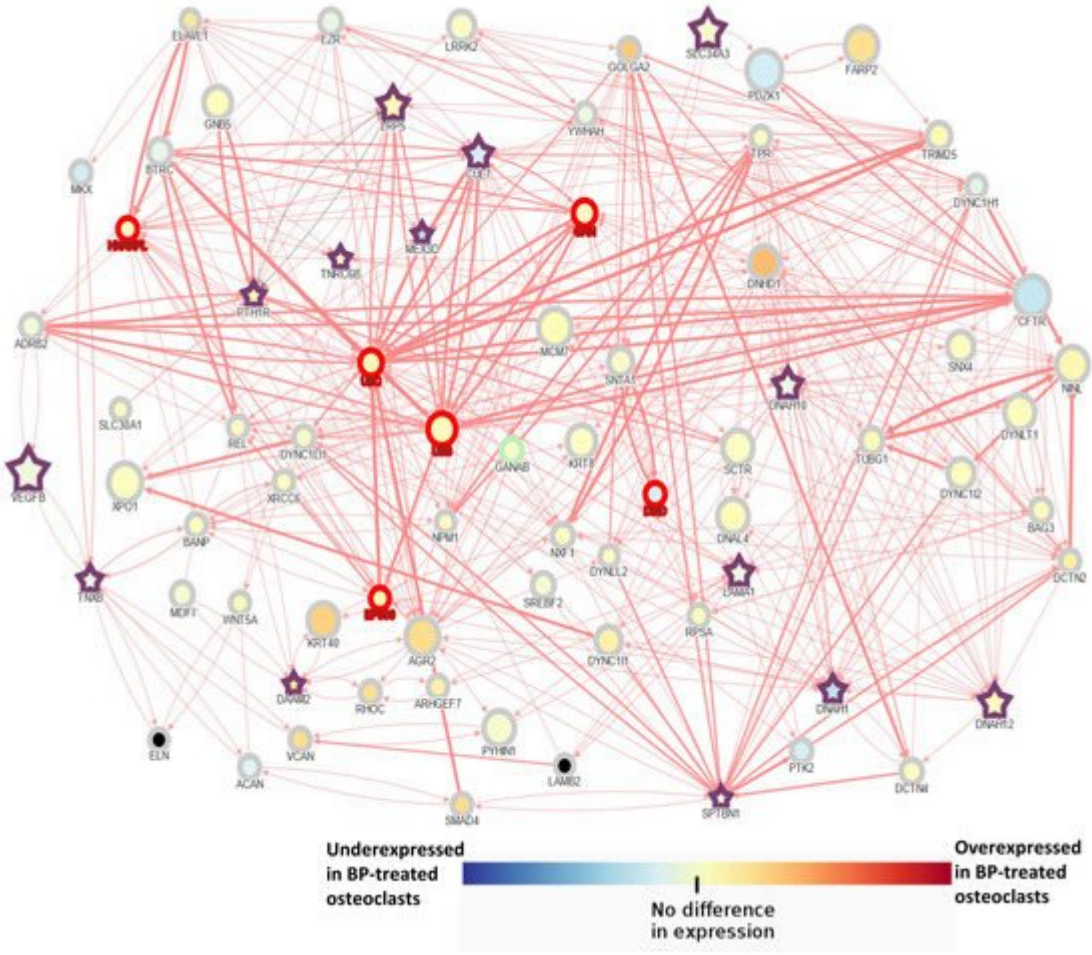


Figure 3. Network analysis of bone-related genes mutated in at least 2 AFF patients using the AFFNET tool. The shortest path interactions among candidate genes were displayed. Purple stars indicate candidate genes. The size of the nodes is determined by observed/expected loss of function score in the gnomAD database. This score is the ratio of the observed and expected loss of function variants in a particular gene. This score provides insight into how tolerant a gene is to loss of function variation. The red border tags genes that are outlier in the constrain metrics for LoF or missense variants according to gnomAD. Node color represents their expression depending on the expression data obtained in the GSE63009: Osteoclastic precursor cells treated or not with bisphosphonates (alendronate or risedronate) during their differentiation into mature osteoclasts.

Besides, a very complex network with multiple interactions among candidate genes and with the other mutated genes (**Table 1**) is displayed when all possible interactions were included in the analysis. Finally, interactions of mutated genes overlapped between the present study and the previous study with 3 sisters with AFF (**Figure 2**) were also explored. No direct interactions were found among these genes even though they were interconnected through one intermediate gene. One gene, *LURAP1L*, had no interactions with other genes. The *MEX3D* gene, which is mutated in 2 patients plus the 3 sisters, is under expressed in BP-treated osteoclasts.

The main conclusion of this study is that AFF may present a multigenic background, specific to each patient, in which an accumulation of susceptibility variants may lead to a predisposition to BP-related AFF. Our analysis suggested that Wnt signaling may play a relevant role in the BP-related AFFs as half of the patients had mutations in a gene of this pathway. In silico analysis suggested a complex interaction network among the different mutated

genes as well as a biological enrichment for cytoskeleton and cilium organization. WES analysis provided evidence to support the hypothesis that several genes and their interactions may be involved in the development of AFF, and, along with BP treatment and, in some cases, glucocorticoids, they may trigger the perfect storm.

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