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COMMENTARY

New GWAS signal on 7q31: which gene is a culprit?

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Two recent studies published in PloS Genetics[1,2] marked the culmination of a barrage of recent studies focusing on some new functional genetic variant(s) of human chromosome 7q31.31, which strongly correlate with bone mass and which have been suggested to be associated with osteoporotic fracture. However, despite relatively strong association signals produced by these variants, the 'culprit' gene hasn't yet been identified, which leaved the underlying biological mechanisms unclear; therefore, potential applications of the reported findings remain uncertain.

The variant region on 7q31 became first known as being linked to the total hip and femoral neck bone mineral density (BMD) (LOD scores =4.15 and 3.09, respectively) in adult men, as reported in the Amish Family Osteoporosis Study.[3] Yet, this signal was not evident in a large meta-analysis of BMD linkage studies.[4] Since advent of the genome-wide association studies (GWAS), the variant in this region has consistently been associated with various skeletal phenotypes, in additional ethnic groups, in women, men and children. First, in a Korean study of 8842 men and women from population-based cohorts (average age ±s.d., 52.2±8.9 years old), rs7776725, an intronic single-nucleotide polymorphism (SNP) in the *FAM3C* gene, was associated with bone ultrasound measures (speed-of-sound) of the distal radius ($P=1.0*10^{-11}$), tibia ($P=1.6*10^{-6}$) and heel ($P=1.9*10^{-10}$).[5] This signal was replicated most recently in two samples of adult Caucasians, both women and men, by Zhang *et al.*[6] Their best association of variant rs7776725 was with dual x-ray absorptiometry-measured wrist ultra-distal radius BMD (combined $P=1.42*10^{-16}$). This SNP was also associated with BMD at other clinically relevant skeletal sites—the hip, spine and whole body.

In the largest GWAS meta-analysis of BMD to date, performed by the GEnetic Factors for OSteoporosis (GEFOS) consortium (involving 32 961 adult individuals of European and East Asian ancestry[7]), the 7q31.31 signal was among the 56 SNPs, which were further replicated in an additional 50 933 independent subjects. SNP rs3801387 was associated with both lumbar spine ($P=1.35*10^{-16}$) and femoral neck BMD ($P=4.23*10^{-14}$). This SNP was also found to contribute to the risk of low-trauma fractures in a massive sampling of 31 016 cases and 102 444 controls (OR 1.06 (95% CI, 1.04–1.08), $P=2.70*10^{-7}$).[7]

Most recently, two publications in PloS Genetics[1,2] reported on the same region. In one, Zheng *et al.*[2] analyzed SNPs associated with cortical bone thickness and forearm BMD in relatively modest-size discovery sample from several cohorts (5878 and 5672 European subjects, respectively). They reported on associations of a missense SNP (Thr.Ile; rs2707466) located in the wingless-type MMTV integration site family member 16 (*WNT16*) gene with cortical bone thickness ($P=6.2*10^{-9}$) and of SNP rs7776725 in *FAM3C*, with forearm BMD ($P=8.5*10^{-15}$). They then evaluated the association of these SNPs with forearm osteoporotic fracture in 2023 cases and 3740 controls; the odds ratios were 1.22 ($P=7.2*10^{-6}$) and 1.33 ($P=8.6*10^{-9}$), for *WNT16* and *FAM3C* SNPs, respectively. Furthermore, another SNP near *WNT16* gene was associated with the total body BMD in a study testing children from different ethnic backgrounds ($n=2660$, mean age=6.2±0.28 years).

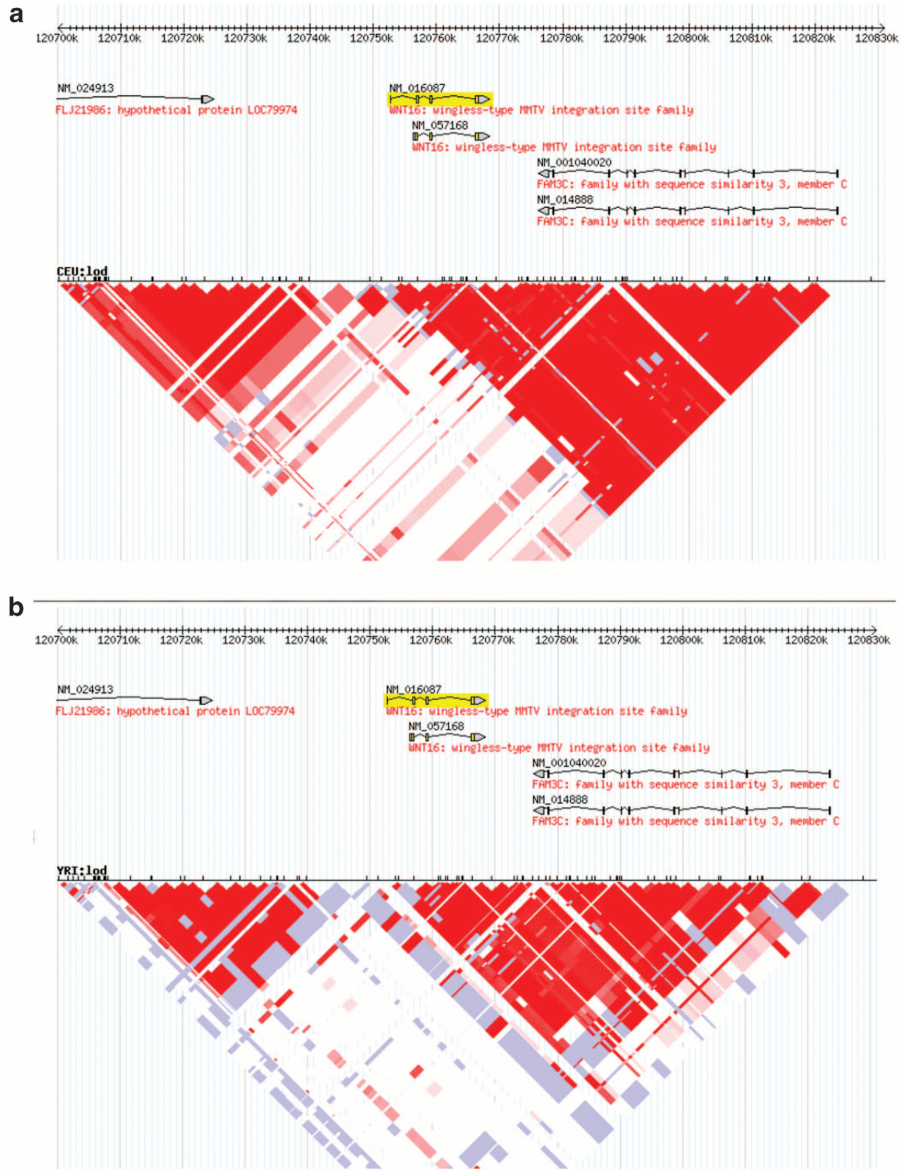

Figure 1 (**a**) Linkage disequilibrium in the 7q31.31 region (Utah residents with Northern and Western European ancestry from the Human Polymorphism Study Center (CEPH)). HapMap data release 27, Phase II + III (Feb. 2009), on National Center for Biotechnology Information (NCBI) assembly B36, dbSNP b126. (**b**) Linkage disequilibrium in the 7q31.31region (Yoruban in Ibadan, Nigeria). HapMap data release 27, Phase II + III (February 2009), on NCBI assembly B36, dbSNP b126.

These findings were replicated in a sampling of adults from the UK and older adults from the Netherlands (joint meta-analysis $P = 1.1 \times 10^{-15}$).[8]

Thus, these studies point to two genes that might generate the signal, which is seemingly responsible for a slew of bone phenotypes. However, the picture became even more complicated when a third gene was suggested to be associated with these phenotypes, by conditioning on (adjusting for) the lead genetic variant. As a result, in the GEnetic Factors for OSteoporosis meta-analysis, the conditional analysis of SNP rs3801387 provided significant evidence of a secondary signal at rs13245690,[7] an SNP located in the intron region of *C7orf58* (chromosome 7 open reading frame 58, a.k.a. *FLJ21986*), with a measure of linkage disequilibrium (LD) with SNP rs3801387 of only $r^2 = 0.028$. Similarly, conditional analyses on the same top SNP revealed a second signal for total body BMD, mapping to *C7orf58*.[1] In the GWAS for cortical bone thickness, conditioning for rs2707466 at the *WNT16* locus also resulted in an additional signal located in *C7orf58*.[2]

Thus, the question which of the three genes is responsible for the association, still remains. The answer lies partly in the design of GWAS, which is based on LD between the analyzed markers and the rest of the variants in the region. When looking at the LD in the 7q31.31region (**Figure 1a**), one can discern one large LD block surrounding *WNT16/FAM3C*, but a weaker correlation between *WNT16/FAM3C* and *C7orf58* alleles. This observation stands in line with the two signals in the region, which seem to be independent of each other.

Traditionally, genes in newly-discovered regions are analyzed by perusing all existing knowledge, in order to establish their candidate status for a specific phenotype. However, this approach was not effective in the case of *WNT16/FAM3C*, as

WNT16 might be a stronger candidate than *FAM3C*, as many members of the Wnt pathway are associated with BMD.[9] Functionally, Wnt16 is involved in specification of the sclerotomal somite compartment, which houses vertebral and vascular smooth muscle cell precursors and has been proposed to signal via the non-canonical pathway,[10] which is conserved in vertebrates. Much less is known about *FAM3C*, which belongs to a family of cytokine-like proteins comprised of *FAM3A*, *FAM3B*, *FAM3C* and *FAM3D* and is ubiquitiously expressed. Of note, *C7orf58* has recently undergone more thorough bioinformatic analyses and has been renamed cadherin-like and PC-esterase domain containing. The cadherin-like and PC-esterase domain containing gene is conserved in the vertebrates, from zebrafish to chimpanzee.

The next step was thus to explore gene–phenotype associations using newly emerged molecular resources. Medina-Gomez *et al.*[1] examined the correlation between gene expression transcript levels derived from iliac bone crest biopsies and BMD levels in Norwegian women.[11] They identified significant correlations of transcripts from *C7orf58* and *WNT16,* but not *FAM3C*, with donor BMD measurements. Furthermore, mice with a gene deletion of *Wnt16* (knockout or Wnt16$^{-/-}$), generated at Lexicon Pharmaceuticals appeared healthy, with normal body weight, body composition and femoral length[1] and with no discernible growth/morphological defects. Yet, male *Wnt16*$^{-/-}$ mice had a slightly reduced cortical thickness and polar moment of inertia (–16%, $P<0.001$), while female *Wnt16*$^{-/-}$ mice had both substantially reduced cortical cross-sectional area, cortical thickness and polar moment of inertia (−36%, −27% and −55%, respectively, all $P<0.001$).[2] In three-point bending tests, measures of both femoral and tibial strength (stiffness, maximal force to breakage and work to failure) were similarly reduced by 43–61% in Wnt16$^{-/-}$ female mice, when compared with wild-type animals.[2] Both studies[1,2] failed to observe any modifications of skeletal phenotype in three independent knockouts of mouse *Fam3c*. Here to note that a Finnish group reported, at the ASBMR 2010 meeting in Toronto, of reduced tibial trabecular bone content in *Fam3c*$^{-/-}$ mice,[12] when compared with wild-type mice at the age of 3 months, due to fewer trabeculi. *Fam3c*$^{-/-}$ mice were generated by utilizing a commercial gene-trap cell clone and were found to have otherwise normal appearance, behavior and fertility.

There are at least two lessons derived from these GWAS-driven discoveries. First, the confluence of GWAS results from a large GEnetic Factors for OSteoporosis study with those of smaller-scale studies,[1,2,5] highlights the trade-off between the statistical power advantage of large sample sizes vs the advantages of homogenous samples (due to age, geography) and better defined phenotype (such as peripheral quantitative computed tomography-measured cortical bone). Secondly, conditioning on the top regional signal sometimes provides unexpected rewards. This semi-serendipitous discovery that *C7orf58 (CPED1)* variants have a role in osteoporosis, represents a good example of the manner in which genome complexity should be deciphered. Differences in LD between the European and African gene pools are expected to provide further direction in fine-mapping the phenotype–gene association. As follows from **Figure 1b**, the African haplotype blocks are shorter, and thus more numerous, therefore, if the association signal replicates in the African-descent samples, shorter LD blocks would assist in pin-pointing a single responsible gene. Then, in-depth re-sequencing studies and functional assays will be required to elucidate the underlying causal variant. Biological pin-pointing of the signal in terms of the gene coding and/or regulatory region, is necessary to enable any clinical application of this discovery toward diagnostics (such as using the gene product as a biomarker) or therapy (developing target-based drugs) of osteoporosis.

Conflict of Interest

The author declares no conflict of interest.

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