The Role of Genomic Copy Number Variation (CNV) in Osteoporosis

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Declaration

This thesis is my own original work and does not incorporate the material of others without proper acknowledgement and appropriate referencing.

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List of Abbreviations

Abbreviation	Definition
α	Alpha
B	Beta
κ	Kappa
n	Nano
µ	Micro
ABI	Applied Biosystems by Life Technologies
ABS	Australian Bureau of Statistics
aCGH	array-Comparative Genomic Hybridisation
ALP	Alkaline Phosphatase
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
APC	Adenomatous Polyposis Coli
AXIN1	Axin-1 gene
BAC	Bacterial Artificial Chromosome
BMD	Bone Marrow Density
Bp	Base pairs
CN	Copy Number
CNV	Copy Number Variant
C_T	Cycle Threshold
COLIA1	Collagen Type I Alpha 1 gene
DGV	Database of Genomic Variants
df	Degrees of Freedom
DvI	Dishevelled
DXA	Dual-energy X-ray Absorptiometry
EDTA	Ethylenediaminetetra-acetic Acid
ESR1	Oestrogen receptor 1 gene

FBAT	Family-Based Association Tests
FRET	Förster Resonance Energy Transfer
Fz	Frizzled
H1RNA	RNase P RNA component H1
gDNA	Genomic DNA
GSK-3	Glycogen Synthase Kinase-3
GWAS	Genome-Wide Association Study
HCl	Hydrochloric Acid
HGA	Human Genome Assembly
IF	Interleukin
LRP5	Low-density Lipoprotein Receptor-related Protein 5
MAF	Minor Allele Frequency
MGB	Minor Groove Binder
MSC	Mesenchymal Stem Cells
NaOH	Sodium Hydroxide
NHS	National Health Survey
NTC	Non-Template Control
OPG	Osteoprotegerin
ORF	Open Reading Frame
PTH	Parathyroid Hormone
qPCR	Quantitative Real-Time Polymerase Chain Reaction
Q-Q	Quantile-Quantile
RAB11FIP3	RAB11 family interacting protein 3 (class II) gene
RANK	Receptor Activator of Nuclear factor κB
RANKL	Receptor Activator of Nuclear factor κB Ligand
RFLP	Restriction Fragment Length Polymorphism
RPPH1	RNase P RNA component H1 gene
RUNX2	Runt-related gene 2

SCGH	Sir Charles Gairdner Hospital
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SOST	Sclerostin
SPSS	Statistical Package for the Social Sciences
TF	Transcription Factor
TGF-β	Transforming Growth Factor-Beta
TNF-α	Tumour Necrosis Factor-Alpha
Tris	Trizma Base
VDR	Vitamin D Receptor gene
VNTR	Variable Number Tandem Repeat
UCSC	University of California Santa Cruz

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Abstract

Copy number variation (CNV) is a relatively novel source of genetic variation, involving the duplication or deletion of segments of genomic DNA (gDNA) sequence, thereby changing the original number of DNA copies. It is currently gaining widespread recognition from the scientific community, and it is anticipated to play a major role in the aetiology of human diseases. However, the extent of its contribution to phenotypic diversity, in terms of individual susceptibility to disease, remains to be elucidated. Nonetheless, recent studies have indicated that common complex disease phenotypes, such as osteoporosis, might be highly susceptible to CNV.

Osteoporosis is a common and debilitating skeletal condition, imposing significant clinical and socioeconomic consequences. The disease is characterised by fragile bones that are susceptible to fracture due to deregulated bone remodelling, where bone loss exceeds bone formation. Being a common complex disease, osteoporosis risk is largely determined by the effect of environmental factors on genetic variants. Moreover, identification of the genetic variants associated with osteoporosis is widely anticipated for the contribution it will make towards the development of improved measures of disease intervention.

Recent genome-wide association studies (GWASs) have identified that the genes oestrogen receptor 1 (ESR1) and Axin 1 (AXIN1) potentially play major roles in bone regulation. In addition, evidence highlights their involvement in key biological processes that regulate bone turnover. Specifically, ESR1 mediates the response of bone marrow-derived cells to oestrogen and it has been demonstrated that oestrogen inhibits bone loss, while AXIN1 inhibits *Wnt* signal transduction and it has been demonstrated that *Wnt* proteins promote bone growth. Furthermore, several large-scale analysis projects firmly implicate genetic

variations of both genes with bone marrow density (BMD), which is the surrogate phenotype of osteoporosis. Therefore, ESR1 and AXIN1 are both recognised candidates for the genetic regulation of osteoporosis risk.

This study investigated the potential effect of two novel CNVs of the genes ESR1 and AXIN1, Variant_4512 and Variant_4912, respectively, in relation to BMD in a population cohort study of Caucasian women, between the ages of 18 and 83, from Australia and the UK. Subjects were genotyped for both CNVs, respectively, using real-time quantitative PCR (qPCR) combined with TaqMan chemistry, and the copy number (CN) quantitation software, CopyCaller. Subjects were then examined for evidence of association between both CNVs and three different BMD phenotypes, 1) raw measurement (g/cm^2), 2) age-adjusted Z-score, and 3) controlled for several covariates, at three common skeletal locations of osteoporotic fracture, 1) lumbar spine, 2) total hip, and 3) femoral neck.

This study confirmed the presence of ESR1 CNV and AXIN1 CNV in the analysed subject cohort, as indicated by the observation of three distinct CNV genotypes for each, representing CN loss (CN1) and CN gain (CN3) from the expected wild-type CN in the human diploid genome (CN2). This study found no evidence of association between both CNVs and BMD ($p = > 0.05$) in the analysed subject cohort. Therefore, the hypothesis tested in this study, that CNV is associated with BMD, was not supported. As a result, it would appear that the ESR1 CNV Variant_4512 and the AXIN1 CNV Variant_4912 are unlikely to play a major role in the pathogenesis of osteoporosis in Caucasian women. However, replication studies and further research would be required to accurately validate this, since this study was subject to numerous limitations which may have influenced the findings, such as low statistical power, technical difficulties, limiting experimental reagents, and time constraints.

In addition, there is evidence from previous studies implicating intron 1 and the 5' end of ESR1 and intron 2 of AXIN1 with BMD. Variant_4512 and Variant_4912 encompass the 5' end of their respective genes, thereby implicating the promoter sequence and regulatory elements, which in turn implicates the control of gene expression. Therefore, despite the lack of statistically significant findings in this study, the ESR1 CNV Variant_4512 and the AXIN1 CNV Variant_4912 both still remain as promising candidates for involvement in BMD and the risk of osteoporosis. Moreover, other CNVs in the same genomic regions may also be relevant for future research.

Further research would benefit from addressing the potential effect of environmental risk factors on CNV. It is possible that the ESR1 CNV Variant_4512 may be modified in an environment-specific manner, which influences its effect on BMD, as indicated by the almost statistically significant association between ESR1 CNV and BMD observed in this study when controlled for covariates at the femoral neck ($p = 0.052$). Moreover, previous studies highlight that the majority of known genes subject to CNV are not even located within the identified region of genomic variation, and also that osteoporosis may be more susceptible to genetic variation affecting the CN of non-coding regions. Therefore, further research should also focus on gene expression studies to determine whether the ESR1 CNV Variant_4512 exerts position effects on the transcriptional control of another gene, which may in turn be the primary gene associated with osteoporosis.