

## **Genetic Markers of Decreased Bone Mineral Density in Cystic Fibrosis**

### **Patients**

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The average life expectancy of persons living with cystic fibrosis has increased significantly during the last decades. As a result new non-respiratory complications such as liver disease, diabetes and male infertility come to the fore and become important clinically for these patients. One such problem is low bone mineral density (BMD) in CF patients, first reported in 1979. Nowadays problems with deteriorating bone quality and quantity are a major problem in adolescents and adults with CF.

### **Decreased bone mineral density in CF patients**

According to some studies, 69% of CF patients suffer from increased bone loss and decreased bone formation (osteopenia), and in 57% severe bone loss occurs. The pathogenesis of low BMD in CF patients is still not certain. According to detailed studies on how bones grow and re-grow, and the cells and nutrients that are involved in bone turnover, both reduced bone formation and accelerated bone resorption play an important role in CF bone disease. Studies of BMD in CF patients show an increase in bone diseases with age, and with the severity of lung disease. The pancreatic insufficiency that is seen in about 80% of CF patients can lead to low levels of calcium, essential lipids and vitamin D, all causing depletion of factors essential for bone growth and remodeling. Disorders affecting bone formation in individuals with CF may also result from chronic pulmonary infections, diabetes, reduced physical activity, delayed puberty, or treatment with steroids or antibiotics.

It was noticed that some small CF children have lower BMD in early life than their healthy peers. There is also evidence that CF patients with F508del mutation in the *CFTR* gene have lower BMD when compared to those with other mutations. The mechanism of

action of this effect is not understood, but these facts suggest that genetic factors influence bone mineral density in CF patients. Two questions remain unanswered: is the CFTR gene expressed on the surface of osteoblasts (bone building cells) or osteoclasts (bone resorption cells), and what is the influence of the F508del mutation on the activity of these cells.

### **Genetic background of decreased bone mineral density**

Decreased bone mineral density has a strong genetic component. Evidence from twin and family studies indicate that the phenotype of decreased BMD is determined by both genetic and environmental factors, and by interactions between them. It is suggested that mutations and/or polymorphisms in many different genes can cause disorders in BMD. Mutations in just a single gene probably do not cause the disease phenotype. Moreover, each mutation and polymorphism in genes that influence BMD can be a risk marker for disorders of bone metabolism. Such gene changes may cause either changes in the sequences of the proteins coded by the gene, or deregulation of gene expression. Any intronic changes, or changes in neighbouring regions of the genome, can be used as gene markers if they are in linkage disequilibrium with identified markers for BMD.

Searching for genes the alleles of which might be responsible for reduced BMD, one should take notice of genes that are regulators of bone metabolism, genes encoding bone matrix components and genes encoding calciotropic hormones and their receptors (Table 1). The most extensively investigated are mutations and polymorphisms in *Collagen Type I Alpha 1 (COL1A1)* and in the *Vitamin D Receptor (VDR)* genes.

*COL1A1* gene codes for collagen type I alpha 1, the major protein component of bone matrix. Alterations in *COL1A1* production and structure lead to abnormal bone matrix, osteopenia and fractures. The *Ball* [1] (intron1) polymorphism alters binding of a protein (Sp1) that regulates the expression of this very important gene. Sp1 can no longer bind well to its recognition site on this stretch of DNA, and as a result there is disturbance in *COL1A1* gene copying, collagen protein production and hence the strength and properties of bone. Other DNA changes in this gene also play an important role in regulation of BMD, and can predict osteoporotic fractures.

The *VDR* gene encodes vitamin D receptor, which is the major regulator of calcium and bone metabolism. Vitamin D, acting through VDR, controls intestinal calcium absorption, and bone growth (osteoblastic) and remodelling (osteoclastic) activities, PTH production and

kidney hydroxylation of one form of vitamin D. Some of the changes that have been found in this gene probably lead to functional changes in the VDR protein, and have an effect on the level of the bone hormone osteocalcin in the blood, and hence on BMD. The *VDR* gene is a key factor modulating calcium and bone mineralization.

Table 1. Overview of genes associated with decreased bone mineral density

<b>Gene symbol</b>	<b>Gene product</b>
<b>Calcitropic hormones and receptors</b>	
<i>VDR</i>	Vitamin D receptor
<i>ESRA</i>	Estrogen receptor alpha
<i>ESRB</i>	Estrogen receptor beta
<i>CALCR</i>	Calcitonin receptor
<i>CALC</i>	Calcitonin
<i>PTH</i>	Parathyroid hormone
<i>GCCR</i>	Glucocorticoid receptor
<i>AR</i>	Androgen receptor
<i>CASR</i>	Calcium sensing receptor
<b>Bone matrix components</b>	
<i>COL1A1</i>	Collagen type I alpha 1
<i>COL1A2</i>	Collagen type I alpha 2
<i>OC</i>	Osteocalcin
<i>ON</i>	Osteonectin
<i>OPN</i>	Osteopontin
<i>AHSG</i>	Alpha 2-HS-glycoprotein
<i>MGLAP</i>	Matrix Gla protein
<b>Bone metabolism regulators</b>	
<i>TGFB1</i>	Transforming growth factor beta 1
<i>IGF1</i>	Insulin-like growth factor 1
<i>IL1B</i>	Interleukin 1beta
<i>IL1RN</i>	Interleukin 1 receptor antagonist
<i>IL6</i>	Interleukin 6
<i>TNFR2</i>	Tumor necrosis factor receptor R2

<b>Miscellaneous</b>	
<i>APOE</i>	Apolipoprotein E
<i>CLGN</i>	Collagenase
<i>CYP19</i>	Cytochrome P450
<i>DBP</i>	Vitamin D binding protein
<i>ADRB2</i>	Beta 2 adrenergic receptor

### **The role of genetic markers in predicting decreased bone mineral density in CF patients**

Several different genes determine bone mineral density. Knowledge of the mutations in genes that are responsible for decreased bone mineral density in cystic fibrosis patients will help to identify individuals who are at risk of bone diseases. Molecular diagnosis of bone turnover disturbances early in childhood and the starting of essential treatment can help to eliminate bone diseases in CF patients.

However, an understanding of the relationship between cystic fibrosis and decreased bone mineral density will require studies that are specific to CF patients, since in addition to the genes known to cause problems with BMD in osteoporosis and other disorders, there is clearly a direct effect of the CF mutation on the severity of the disorder. The typing of candidate genes and analysis of their polymorphic variants should increase knowledge of decreased BMD in cystic fibrosis, and may help to define treatment guidelines.

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[1] The name of each polymorphism is taken from the name of restriction enzymes that differentiate nucleotide sequence enabling allele identification.