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Review Article

Hylonome

Femoral neck fragility: Genes or environment?

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Introduction

The major clinical importance of osteoporosis lies in its association with bone fragility. Osteoporosis is currently defined as an areal bone mineral density (aBMD) value more than 2.5 standard deviations (T-score values) below the population average in young adults[1]. This report focuses on hip fracture because it accounts for approximately two-thirds of the costs of direct medical care for osteoporosis and because the age-specific incidence rises steeply by 30%[2] for every two-year increase in life expectancy.

Genetics and bone mass

The skeletal determinants of osteoporotic fracture risk, such as aBMD, bone geometry and bone turnover, are all subject to strong genetic influences. It has been estimated from twin studies that 60–85% of the variance in aBMD is genetically determined[3], and heritability estimates for other risk factors such as quantitative ultrasound, femoral neck geometry and bone turnover markers range between 50–80%[4,5]. Although a family history of fracture is a risk factor[6], the heritability of fracture itself is relatively low (25–35%)[7], reflecting both the importance of fall-related factors and the possible effects in the pathogenesis of fracture.

Major advances in our knowledge about the genetic determinants of osteoporosis include the discovery of genes responsible for monogenic bone diseases associated with abnormal bone mass[8]; the identification of quantitative trait loci for bone mass in the general population and in mice; and the characterisation of several candidate genes for osteoporosis. To date, over 40 candidate genes have been identified as having an important effect on bone mass[9,10]. These range from genes encoding calciotropic hormones (e.g., PTH and calcitonin), hormone receptors (e.g., those for VitD and estrogen), bone matrix proteins (e.g., Col1A1 and osteocalcin) local regulatory factors (e.g., TGF_, IGF-1 and osteoprotegerin) to those that may alter bone cell differentiation (e.g., PPAR_ & LRP5). Although there are concerns regarding the statistical power of a number of published studies, with the resultant reports of both positive and negative findings for a particular gene, it is clear that a few of these genes have consistent effects on bone mass and possibly bone fragility. In the case of the haplotypes for COL1A1 and ERα, there appears to be dissociation between gene effects on fracture risk and on BMD. What is generally lacking, however, are plausible biological mechanisms whereby some genes might contribute to femoral neck fragility. Partly to address this issue we now review our case-control studies into the aetiology of hip fracture that our group has initiated over the last decade and indicate where some of these genetic factors might influence the changes in bone geometry, microstructure, remodeling and material properties associated with femoral neck fragility.

Pathogenesis of femoral neck fragility

Although aBMD is an important determinant of fracture risk it only accounts for about 30% of the risk of hip fractures[11]. Other factors considered to play a role include bone turnover, bone geometry, bone microstructure and the material properties of the remaining bone along with 'non-skeletal' risk factors that influence the risk of falling, such as muscle strength, balance and visual acuity.

Bone geometry

Much of the strength of whole bones resides in the cortex. This is particularly the case in long bones such as the femur and it has been argued that extremity fractures including those of the femoral neck originate in cortical bone rather than the spongiosa[12]. This shift in emphasis is supported by

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both experimental evidence[13] and finite element analysis[14] indicating that the femoral neck cortex supports at least 50% of the load borne by the proximal femur. During normal gait, peak compressive stresses occur in the inferior neck cortex. These change during a sideways fall onto the greater trochanter; and peak compressive and tensile stresses at impact occur in the supero-posterior and infero-anterior cortex, respectively[14,15]. Clearly, genes which influence the initial geometry of the femoral neck[16] and those which influence its response to the lifetime range of physical activity will be important in determining the strength of the femoral neck during a fall.

Bone remodeling and microstructure

Cortical remodeling demonstrated marked regional variation with the anterior region of the cases having significant elevations in the proportion of both forming and resorbing canals[17]. Moreover, this remodeling was not randomly distributed within the cortex but was anatomically clustered[18] with approximately 60% of canals with an osteoid seam within 375μm of another canal with an osteoid seam. Cases had more clusters than controls. Further studies have demonstrated that this clustering occurs at other cortical sites such as the femoral shaft[19] and the ilium and that it is not age- or gender-dependent[19]. Pilot data suggest the possibility that both underloading of bone and the initial rate of bone expansion may play a role in the formation of remodeling clusters but this requires more extensive investigation. These studies suggest that genes involved in the response to loading (estrogen receptor; and the rate of circumferential growth (GH, IGF-1 gonadotropin receptors) are likely to be those that will influence cluster formation.

Histomorphometric and pQCT analysis of whole cross-sections of the femoral neck taken from cases of intracapsular fractures have clearly demonstrated a marked reduction in both cortical width[20,21] and porosity[22] compared to age- and gender-matched post-mortem controls. These changes were regionally dependent with the most affected cortices being the anterior, infero-anterior and infero-anterior regions, while the supero-posterior cortex is the thinnest in both cases and controls. Cases had an approximate doubling of the density of so called "giant" pores. These represented only the top 0.5% of the size distribution of pores in the control biopsies. The morphology of these giant canals suggests that they could be the result of the merging of clusters during the resorption phase of BMU remodelling. Further analysis of the giant canals suggests that over 80% of them have more than one cement line, strengthening the idea that some form of failure to limit bone resorption results in the merging of individual canals to form "composite" canals. Immunocytochemical studies on the expression of eNOS and nNOS in osteocytes indicates that fracture cases have about a 50% reduction in osteocytes expressing both NOS isoforms and that these are generally seen towards the periphery of individual osteons[23,24]. As NO is inhibitory to osteoclastic resorption[25,26], this could imply that the failure to express eNOS results in excessive bone resorption leading to the merging of individual canals to form a composite osteon.

Material properties

The elasticity of bone and thus its resistance to fracture is related to its degree of mineralisation[27,28]. Both hypo- and hyper- mineralisation and increased heterogeneity decreases bone strength. Femoral cortical bone with an ash content above 60% by weight, has a decreasing ability to absorb impact energy and is increasingly liable to fracture because microfractures can more readily propagate through bone that is highly mineralised[27,29]. The mineral concentration in any microvolume of bone normally increases asymptotically with the **tissue** age until it is removed by osteoclasis. Thus, as bone turnover and replacement tends to reduce with **chronological** age, there may be a net increase in the proportion of bone micro-volumes that have a higher density[29-31].

We have measured the mineralization density in femoral neck biopsies from female hip fracture cases and controls using the quantitative back-scattered electron imaging approach developed by Boyde and colleagues[30,32]. Over the whole biopsy the level of mineralization was lower in the cases than the post-mortem controls[33]. In both cases and controls mineralization was higher in the inferior (compressive) region compared with the superior (tensile) region. Mineralization was lower in all regions of the cases. New bone formation was higher in the anterior, inferior and posterior regions of the cases. However, there was no relationship between % osteoid bearing canals and the mean level of cortical mineralization when assessed, either over the whole biopsy or on a regional basis. Thus, unlike the study of mineralization at the ilium and its relationship to vertebral fractures[34] matrix mineralization is reduced in the femoral neck of cases of intracapsular hip fracture. Unexpectedly, in view of the likely role of mild to moderate vitamin D deficiency osteopathy, this decreased mineralization was independent of osteoid surface. This raises the possibility that alterations in the bone matrix such as excessive glycation or changes in the composition of the collagen fibrils might play a role in the aetiology of hip fracture. For instance, osteoporotic fractures have been associated with an increased presence of a polymorphism in the SP1 binding site of the COL1A1 gene, the presence of which results in a lower yield strength[35] and a lower mineralisation density (Ralston & Klaushofer; personal communication).

To examine if the changes in mineralization affected the hardness of the femoral neck cortex, we used a micro-indentation technique on a separate group of cases and controls[36]. Not surprisingly, interstitial bone was harder than osteonal bone and hardness was related to the proportion of canals with new bone formation. There were marked regional differences in the hardness of both osteonal and interstitial bone with the posterior and inferior regions having higher hardness values than the anterior and superior regions. Fracture cases had significantly lower hardness values in the

anterior and inferior regions for osteonal bone and in the anterior region for interstitial bone. However, even after adjustment for the indices of remodeling the case-control differences remained again suggesting that some of these differences in material properties were inherent rather than the result of increased bone turnover reducing the bone age.

Summary

As with other diseases that show exponentially increasing rates, hip fracture probably requires multiple prior events to become manifest. It is common ground that there are genetic, environmental, lifestyle and perhaps dietary determinants of risk of osteoporotic fracture as well as interactions between them. The key to secondary prevention is the understanding of how these components can be integrated into an effective assessment of the major risks of hip fracture after a previous fracture has occurred[37]. The key to primary prevention is to understand both the pathological and physiological basis of hip fragility. It is not unreasonable to suppose that in a western lifestyle our limited and stereotypic patterns of locomotion from middle age onwards may offer considerably less protection than, for example, the more physically demanding activity of subsistence farming[38-40].

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