3. Estrogen receptor status and transcriptome profiling of genes regulated by 17-beta estradiol in human osteoblasts derived from idiopathic scoliosis patients.

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Adolescent idiopathic scoliosis (AIS) is the most common form of spinal deformity affecting up to 4% of schoolchildren worldwide. The etiology and molecular mechanisms of AIS are not clear; currently the consensus on AIS is of a multifactorial etiology, but the involvement of genetic factors is widely accepted. Several physiopathological, clinical and molecular observations suggest that hormones such as melatonin, estrogens and growth hormones play a critical role in bone mass acquisition and consequently in the progression of AIS, but the precise mechanisms involved are yet unclear. The role for estrogen seems possible due to its interaction with many factors that influence the development and progression of this spinal deformity.

Additionally, estrogens impact bone remodeling and growth as well as bone acquisition, all of which are affected in AIS. To study the role of estrogen in AIS, six unrelated individuals with AIS and 6 controls (non-AIS individuals), all French Canadian females from Quebec were studied. Gene expression profiling was preliminarily investigated by microarray analysis in RNA samples from Osteoblasts derived from control (non AIS) and AIS patients. Osteoblasts were cultured for 16 h without or with increasing amounts of Estradiol .

Data analysis was performed in R version 2.10.1 (Bioconductor packages oligo and limma). Selected genes with change fold greater than 1.5 were further investigated by RT-qPCR. Microarray analysis revealed several genes that are differentially regulated in AIS osteoblasts compared to control. Many of these genes are involved in different physiological signaling pathways. When we compared the transcriptome profiling of estrogen –regulated genes between non-AIS and AIS osteoblasts, several genes were up- and down-regulated in response to estrogen. W e considered five genes for further anaylsis.These genes showed the most modification upon estrogen treatment and 3 of these genes were previously associated with AIS.Insilico analysis,show that these genes have several estrogen reponse elements in their promoters which confirm the fact that these genes are estrogen regulated. We successfully cloned these genes in PGL3 vector which includes a luciferase coding sequence. The luciferase activity will be measured in the presence or absence of estrogen. More than one gene is likely responsible for AIS, and some of these genes are estrogen–regulated. In the absence of specific causative gene(s) for AIS, our study of gene expression by microarray pointed out putative biological pathways and genes to be carefully investigated.