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THE GENETIC INFLUENCES OF THE DEVELOPMENT OF OBSTRUCTIVE SLEEP APNOEA AND ITS ASSOCIATION WITH LOW BACK PAIN AND LUMBAR DEGENERATION

PhD thesis

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List of Abbreviations

A, h2 - additive genetic effects

AF - Anulus Fibrosus

AHI - Apnoea-Hypopnoea Index

AI - Arousal Index

ASSM - American Academy of Sle Medicine

BMI - Body Mass Index

C, c2 - common environmental effects

CIH - chronic intermitting hypoxia

CPAP - Continuous Positive Airway Pressure

CT - Computer Tomography

DM - Diabetes Mellitus

DNA - Deoxyribonucleic Acid

DZ - Dizygotic

E - other residual effects

ECM - Extracellular Matrix

ECG - electrocardiogram

EEG - electroencephalogram

EMG - electromyogram

eNOS - endothelial nitric oxide synthase

EOG - electrooculogram

EP - Endplate

ESS - Epworth Sleepiness Scale

HIZ - High Intensity Zone

H_2O_2 - Hydrogen Peroxide

ICD - Implantable Cardioverter-Defibrillators

ICSD - International Classification of Sleep Disorders

IL1 - Interleukin 1

IL-1β - Interleukin-1β

IL6 - Interleukin 6

IL8 - Interleukin 8

IL17 - Interleukin 17

LBP - Low Back Pain

MAPK - Mitogen-Activated Protein Kinase

MinSatO2 - minimal oxygen saturation

MMP – matrix metalloprotease

MR – Magnetic Resonance

MRI - Magnetic Resonance Imaging

MZ – Monozygotic

NF-κB - Nuclear Factor-κB

NP - Nucleus pulposus

NO – Nitrogen Oxygen

ODI - Oxygen Desaturation Index

OH - Hydroxyl Radicals

O_2^- - Superoxide Anions

OSA - Obstructive Sleep Apnoea

PI3K - Phosphoinositide 3-Kinase

PGE2 - prostaglandin E2

PKC - Protein Kinase C

PSG - polysomnography

PTH - parathormone

RDI - Respiratory Disturbance Index

REM - rapid eye movement

RERA - Respiratory Effort Related Arousal

RespAI, respiratory arousal index

RMDQ - Roland-Morris Disability Questionnaire

RNA - Ribonucleic acid

ROS- Reactive Oxygen Species

SE - standard error

SPT – Sleep period Time

STIR – Short Tau Inversion Recovery

TEPS – Total End Plate Score

TNF-α – Tumour Necrosis Factor- alpha

TST – total sleep time

TST90% - sleep time spent with oxygen saturation below 90%

UA – Upper Airways

YLD – years lived with disability

1 Introduction

1.1 Obstructive Sleep Apnoea

Sleep is very important in our lives and is crucial for our well-being, health, and daily functioning.[1] Nothing proves the importance of sleep better than the fact that we spend about a third of our lives in sleep. In Europe, the incidence of sleep disorders is between 16.6% and 31.2%, indicating that it is a very common disease in modern society.[2] Sleep disorders are categorized by the International Classification of Sleep Disorders, Third Revision (ICSD – 3), which lists diseases in 7 major categories [3]:

- Insomnia
- Sleep-related breathing disorders
- Central disorders of hypersomnolence
- Circadian rhythm sleep-wake disorders
- Parasomnias
- Sleep-related movement disorders
- Other sleep disorders

Obstructive Sleep Apnoea (OSA) is a common sleep-related breathing disorder, characterised by a partial or total collapse of the upper airways (UA) leading to intermittent oxygen desaturation and frequent awakening[4]. The prevalence of the disease is between 3% and 7%, so it is one of the most common sleep disorders[5]. OSA has day- and night-time symptoms, like excessive daytime sleepiness, loud snoring, observed episodes of stopped breathing during sleep, morning headache, difficulty concentrating during the day, mood changes such as depression or irritability etc., and leads to several complications in the patient's life: daytime fatigue and sleepiness at work or home, cardiovascular problems, and complications with medication and surgery.[4]

1.1.1 The pathogenesis of OSA

The central element of the pathogenesis is the apnoea (the pause in breathing) or hypopnea (decreased breathing) caused by impassable airways, and in parallel the failure of the exchange of respiratory gases. Hypoxia and hypercapnia increase breathing effort through the chemoreceptors, and at the same time cause sympathetic tone enhancement. If the upper airway collapse is not resolved as a result of these changes, then sympathetic activity further increases, leading to awakening (arousal) in the central nervous system.

To correct the displaced blood gas parameters a hyperventilation period occurs, after which the patient returns to the slow-wave stage of the sleep cycle.[6] As sleep deepens, pathological processes are reinitiated and lead to re-hypoventilation and repeated blockage of the upper respiratory tract. As a result of cyclical awakening, the normal sleep cycle is interrupted, and the sleep is fragmented.[6]

The blockage of the upper respiratory tract, meaning the nasal cavity, the pharynx (naso-, oro-, hypopharynx), and the larynx, is the key element in the development of the disease. Any factor that promotes the blockage or obstruction of the upper respiratory tract is an important etiological factor. We can define two main causes for this process: anatomical abnormalities and non-anatomical (such as neurological or muscular) abnormalities .[7]

Anatomical abnormalities or variabilities are among the important factors in the pathological mechanism of OSA. Lumen-narrowing lesions such as tonsillar hypertrophy[8], retrognathia[9], or craniofacial malformation[10] promote the development of OSA. Previous studies have shown that OSA patients' upper airways have smaller cross-sectional areas than those of the healthy control group.[11] Other studies have revealed that the maxillary and mandibular size as well as body length can increase the vulnerability to OSA[12]. Furthermore, parapharyngeal fatty tissue accumulation and increased body weight also decrease the diameters of upper airways, leading to collapse.[13]

The diameters of the upper respiratory tracts, especially the lumen of the pharynx, largely depend on muscle tone, because the pharynx can be described as a fibromuscular structure. At the beginning of sleep, muscle activity diminishes and later increases again to maintain the penetrability of the upper airway. This is a neurocompensatory response that keeps the upper airways open, but this response is often injured or missing in patients with OSA.[14] Other studies have described higher muscle tone in patients with OSA, which decreases with Continuous Positive Airway Pressure (CPAP) therapy.[15] The higher muscle tone likely compensates a narrowing anatomical abnormality of the upper airway; because CPAP therapy maintains the intraluminal pressure during sleep and ensures that the upper airway is open, the muscle tone does not need to be high. Consequently, without CPAP therapy, the normal global muscle tone decreases during

sleep, and existing anatomical abnormalities increases the risk of upper airway collapse. This mechanism is called the loss of wakefulness stimulus.[16]

When a disturbance occurs in the breathing system, the ventilatory drive increases to maintain the airways. This response can be described in the terms of the “loop gain”, which is the ratio of the response of the upper airway (UA) to a ventilatory disturbance, and is comparable to a feedback loop. In OSA, the loop gain is usually higher than one, meaning that the ventilatory drive increases more than the required reduction in ventilation, so the ventilatory control system is unstable. This instability is also important in the pathogenesis.[17]

Muscle tone is strongly influenced by the reflex of the pharynx mechanoreceptors, which respond to decreasing intrapharyngeal pressure by increasing muscle tone. This reflex can be considered as the pharynx's reflex because it also occurs if the brain stem connection is interrupted, and its effectiveness is reduced when the mucosa is anaesthetised.[18] In addition to the reflex described above, the central nervous system also has a direct influence on the tone of the muscles responsible for keeping the pharynx open. Experiments in rats have shown that direct cholinergic pathways run from the brainstem to the musculus genioglossus, and these may be responsible for the differential tone in wakefulness-sleep states.[19] Therefore, the muscle tone of the upper airways is the summary of local factors and central nervous system influences.

Because other protective mechanisms fail, arousal—a brief awakening from sleep—occurs at the end of OSA breathing events in an attempt to restore airflow and correct the blood-gas disturbance. Accordingly, the arousal threshold can be defined as respiratory drive or pressure that is present immediately prior to a clinically defined cortical arousal which can be seen in an electroechephalogram (EEG). This is cardinal in OSA patients because the lower arousal threshold level leads to more awakening, more sleep fragmentation, and several symptoms. In conclusion, impaired neural control, muscle activity, and anatomical abnormalities or variabilities all increase the risk of OSA. (Figure 1)

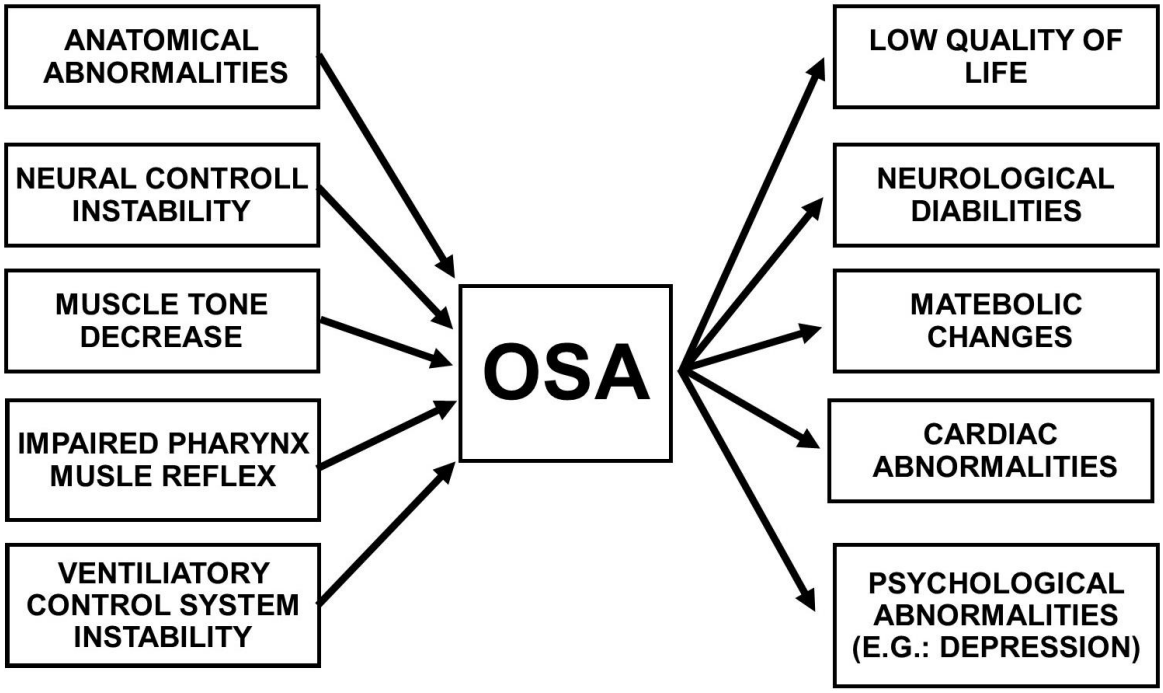

Figure 1: Pathogenetic factors and consequences of OSA.

OSA is a multifactorial, complex disease in which developmental, anatomical, and non-anatomical factors are key elements. As a result of OSA, many other diseases like heart and vascular, metabolic, psychological and neurological diseases are greatly promoted; OSA also has a negative impact on patients' quality of life[4]. *(author's own illustration)*

1.1.2 Inheritance of OSA

Previously, a family study conducted by Patel et al. described the heritability of OSA [20], and later another family study proved that the smallest surface diameter of the oropharynx is a heritable trait.[21] Luqi Chi et al. examined 55 apnetic probands and 55 siblings of probands as controls, and showed in a magnetic resonance imaging (MRI) cephalometric measurement that many parameters like sella–nasion–subspinale angle, saddle angle, mandibular length, lower facial height, mandibular width, maxillary width, distance from the hyoid bone to the retropogonion, and size of the oropharyngeal space are heritable.[22] Because of the common environment of the participants, the family studies have limited ability to isolate the genetic and common environmental factors. Twin studies have a better opportunity to distinguish these factors; they can also take shared environmental factors into account, including the potentially deleterious and

confounding effects. Only a few twin studies have been performed in connection with OSA; one measured the hereditary factors in the background of sleeping[23], and the other estimated the inheritance of OSA to be over 50%.[24] Both studies have restrictions: they collected the data by questionnaire, and the OSA was not confirmed by clinical tests. Furthermore, the severity of OSA was not evaluated by a diagnostic sleep study.

1.1.3 Diagnostic methods of OSA

In general clinical practise the first step in the diagnostic procedure is recording the patient's medical history and symptoms. It is important to ask about the patient's life behaviour, sleep habits, compliance with sleep hygiene rules, the presence of snoring, choking, gasping, sweating, and the appearance of pathological movements during sleep, like restless leg syndrome. It is also important to assess the next day's wakefulness symptoms like drowsiness, fatigue, poor performance, and whether cataplexy may be present in the patient's life.[25] Questionnaires are also a great help in diagnosing OSA and assessing symptoms. Questionnaires such as the EPWORT sleepiness questionnaire (Supplement 1) to assess daytime sleepiness[26] and the STOP-BANG questionnaire, which helps to screen for suspected cases of OSA, are also widely used in daily clinical practice.[27] Other tests like Sleep Apnoea Clinical Score, Berlin Questionnaire, cricomental distance, OSA50, elbow sign questionnaire and American Society of Anesthesiologists checklist are also available and useful test to diagnose OSA.[28] After recording the clinical symptoms, polysomnography or home apnoea testing is necessary to diagnose OSA. The polysomnography is the gold standard method, but it must be performed in a hospital, needs an expensive device and a qualified person. Because of these reasons, polysomnography is an expensive test method with limited availability. Home apnoea testing such as ApnoeLink, which is a cardiorespiratory polygraphy, can be an alternative option. This device measures nasal airflow, oxygen saturation and respiratory effort. The cardiorespiratory polygraphy’s pooled sensitivity was 92% (95% confidence interval 87.5–95%) and the pooled specificity was 54% (41–67.3%).[29] The advantage of these devices is that the test can be performed at home and opposed to polysomnography it is cheaper, making it more widely accessible.

1.1.3.1 The polysomnography

The benchmark objective testing is polysomnography; this is conducted in hospital conditions in a sleep laboratory. The examination aims to differentiate and determine which sleep disorder the patient is suffering from. Polysomnography is performed with a complex, multi-component device that includes an electroencephalogram, electromyogram (EMG), electrooculogram (EOG), pulse-oximetry, airflow measurement, microphone, and in many cases can be supplemented by an electrocardiogram (ECG) and video recording.[25] The purpose of the study of brain function is to isolate sleep cycles and to determine wakefulness. Typically, a 10 or 20 electrode EEG is made during the test. With the help of EOG, the REM phase can be detected easily due to the appearance of rapid eye movements. Furthermore a typical pre-sleep phenomenon—slow rolling eye movement—can also be detected, which helps to determine the onset of sleep. The EMG helps in the determination of the REM period and provides information on the degree of muscle tone in the body. To test the air flow, a nasalis or oral thermistor is placed, which sets inhalation and exhalation with temperature change measurements. It follows that it is unsuitable for measuring partial flow reduction, so it is also necessary to also install a pressure sensor. With a pulse oximeter we can measure blood oxygenation. In OSA, during periods of apnoea and hypopneas a decrease in oxygen saturation can be observed, which is significant in assessing the severity of the disease. In addition, it is recommended to install an ECG to monitor the status of cardiology, to place a microphone for snoring monitoring, and to make a video recording for detecting the patient's night movements. An EMG affixed to the affected muscles (e.g., in restless leg syndrome) is also suitable for the examination of the patient's movements.

1.1.3.2 Calculated indexes in polysomnography

The variables measured during the procedure are represented on graphs on a time axis below each other.

- The Apnoea Hypopnea Index (AHI) is the number of apnoea and hypopnea in an hour. Most studies show that the normal range is below 5 events per hour. By definition, apnoea means the lack of airflow (90% decrease of the nasal airflow), which persists for up to more than 10 seconds. Hypopnea is a more than 30% decrease in airflow lasting for 10 seconds, which is related to ≥ 3% oxygen desaturation or arousal.[25] In clinical practice, AHI is used to diagnose and determine the severity of OSA. If AHI is above then 5 events/hour, but below 15 events/hour mild OSA, if AHI is above 15 events/hour, but below 30 events/hour moderate OSA and if AHI is above then 30 events/hour several OSA was diagnosed.
- The Oxygen Desaturation Index (ODI) is the number of desaturation events (3 percent reduction in blood oxygen saturation) in an hour. The ODI is not the same as the AHI because the latter shows the number of events that cause the desaturation, while the former shows its consequences. In addition, the ODI is also affected by many other factors, such as the state of the respiration system and congestive heart disease.[25]
- The Respiratory Disturbance Index (RDI) can also be interpreted as an expanded AHI, which includes RERA (Respiratory Effort Related Arousal) events. A RERA is a respiratory event accompanied by desaturation and awakening that cannot be described as hypopnea or apnoea. Therefore, the RDI also includes other respiratory abnormalities in the number of events that occur within an hour, so the abnormal population is larger than in the AHI since its normal value is also an event-per-hour figure below 5.[25]
- Arousal Index (AI) is defined as the number of awakenings per hour. Arousals can be classified further by their origins: respiratory, limb movement, spontaneous, and others like respiratory effort–related arousal (RERA) or arousal caused by external stimuli.[30]

1.1.4 Complications

1.1.4.1 Cardiovascular system

Patients with OSA have an increased risk of atrial fibrillation[31] and high blood pressure[31], which increases their overall mortality.[32] Due to periods of hypoxia the normal autonomous hemodynamic response to sleep is disrupted and the global sympathetic activity increases, causing continuous vasoconstriction in peripheral veins. As a result, blood pressure rises, which can be up to 240/130 Hgmm at the end of the apnoea period. At the same time, elevated blood pressure, hypoxia, hypercapnia, and sympathetic activation indicate inflammatory reactions; oxidative stress also increases following the apnoea period. The combination of these factors leads to endothelial dysfunction. These changes also cause platelet activation, which moves the clotting system in the direction of hypercoagulability and promotes the formation of blood clots.[33]

1.1.4.2 Diabetes mellitus (DM)

Increased sympathetic activity in apnoea indicates an increase in adrenergic hormone levels, which reduces insulin sensitivity in organs, thereby contributing to the development of Type II diabetes. OSA has been shown to increase insulin resistance regardless of other factors, which decreases as a result of CPAP treatment.[34]

1.1.4.3 Stroke

A cross-sectional study revealed that OSA has a strong relationship with stroke. As a result of OSA, pathological changes occur in the cardiovascular system, such as spikes in blood pressure, a decrease in blood flow to the brain, insufficiency of its autoregulation, dysfunction of the endothelium, increased risk of thrombosis, and predisposition to atherosclerosis.[33] OSA also plays a significant role in stroke survival, with premature death being higher in OSA patients than in non-OSA patients.[35]

1.1.4.4 Other risk factors

Another study showed an elevation in depression, hypochondriasis, and hysteria in the Multiphasic Personality Inventory scale.[26] More and more data indicate that OSA patients have a poorer quality-of-life. D'Ambrosio et al. used a quality-of-life questionnaire (Medical Outcomes Study Short Form)[6] before and after 8 weeks of CPAP therapy and showed that the treatment increased vitality, social function, and

mental health.[36] Several studies verified that car driving is complicated by OSA; the number of accidents is higher than in the non-OSA group.[37]

1.2 Low Back Pain (LBP)

1.2.1 Connection with Obstructive Sleep Apnoea

Chronic pain is a common symptom in patients with sleep disorders, and people with chronic pain often have difficulties with their sleep.[38] If chronic pain, sleep disturbances, and mood disorders are present at the same time, an umbrella term of fibromyalgia is often used.[39] The link between sleep disorders and chronic pain is related to genetic and neuroendocrine factors, altered sensitivity of pain, and systemic inflammation.(38,40) The prevalence rate of OSA in patients with chronic pain is 32%[41], and the prevalence of excessively sleepy patients with OSA among those with chronic pain is 14%.[42] Low back and neck pain is one of the most common causes of chronic pain, but interestingly previous studies focusing on the cervical spine only reported that OSA was associated with an increased number of cervical fusions and osteophytes.[43]

More recently, cervical spondylosis was reported as a risk factor for OSA.[44] Yang et. al. hypothesised that spondylosis leads to increased collapse potential of the upper airways, as the posterior pharyngeal wall is displaced anteriorly.[44] Curiously, no studies have assessed the lumbar spine region in OSA, although around one-third of patients with chronic back pain had abnormalities in their lumbar regions.[45]. However, there is some evidence that OSA could also lead to spinal deformities directly through chronic intermittent hypoxia.[46] Experimental models show that chronic exposure of anoxia stimulates osteoclast formation and downregulates osteogenic differentiations, promoting bone resorptions.[47] Otherwise the intermitting hypoxia contributes to the early mobilisations of mesenchymal stem cells to blood circulation; these cells are important in the differentiation of osteoblast and endothelial progenitor cells. These changes suggest a reparative mechanism for the hypoxia event. The majority of studies suggest that OSA assists bone resorptions and osteoporosis, but interestingly Sforza et. al. found that bone mineral density in their elderly participants was higher in OSA patients than in healthy ones, which leads us to conclude that OSA is a protective factor.[46] However it is important to note that, this has not been confirmed by any other study since.

Accordingly, there is a high probability that there is some link between the two diseases. Another indirect connection is supposed to be through vitamin D deficiency. In OSA

patients the vitamin D level is lower, and the parathormone (PTH) level is higher than in the control group. After short-term (7 nights) CPAP therapy, a significant increase was found in the level of vitamin D in male participants, but in the female subjects, this change could not be detected. In conclusion, the relevant treatment of OSA may help the recovery of vitamin D homeostasis.[48] Most importantly, intermittent hypoxia may lead to oxidative stress and inflammation in the lumbar discs which could contribute to spinal disc degeneration.[49]

1.2.2 The importance and background factors of low back pain

Low back pain is a very common chronic pain syndrome. The prevalence of the disease is between 15% and 45%, and most studies include the proportion of people who experience this complaint between 70% and 80% during their lifetime.[50] In Hungary, 18,000 out of 100,000 people suffer from vertebral and disc disorders, making it the second most common disease in the country.[51] According to a study conducted in 2013, back pain was the leading cause of the number of years lived with disability (YLD).[52] Low back pain is mostly caused by lumbar degeneration, but other abnormalities are also possible in the background. The most common causes are lumbar intervertebral discs, the apophyseal joints, and sacroiliac joint degeneration; other lumbar abnormalities like intervertebral disc prolapse and endplate (EP) fractures and disc dehydration are also associated.[53] Age, higher Body Mass Index (BMI >30) and female gender are the most common risk factors of low back pain, but other factors like low education level, manual work (manual handling, bending, twisting and whole-body vibration) and bad work conditions (job dissatisfaction, monotonous tasks, poor work relations, lack of social support in the workplace, demands, stress, and perceived ability) also negatively impact the progression of low back pain.[54] Many psychological factors like anxiety, depression, somatisation symptoms, stressful responsibility, job dissatisfaction, mental stress at work, negative body image, weakness in ego functioning, and poor drive satisfaction also promote low back pain.[50] Polatin and colleagues suggest that substance abuse and anxiety disorders precede chronic low-back pain, whereas depression may develop before or after the onset of this type of back pain.[55] In short, low back pain is caused in most cases by lumbar degeneration, but other factors are also important.

1.2.3 Lumbar degeneration

Lumbar degeneration is a complex process that affects the bone, muscle, and joint systems of the spine and appears at the biochemical, cellular, and tissue levels, causing changes in the morphological and functional aspects of the spine. The progression of the pathological process leads to a decreased stability of the spine, which generates back and spinal pain. Structural changes in lumbar degeneration can be divided into 4 categories: small joint degeneration, disc degeneration, bone changes, and ligament and musculoskeletal abnormalities. (Figure 2).

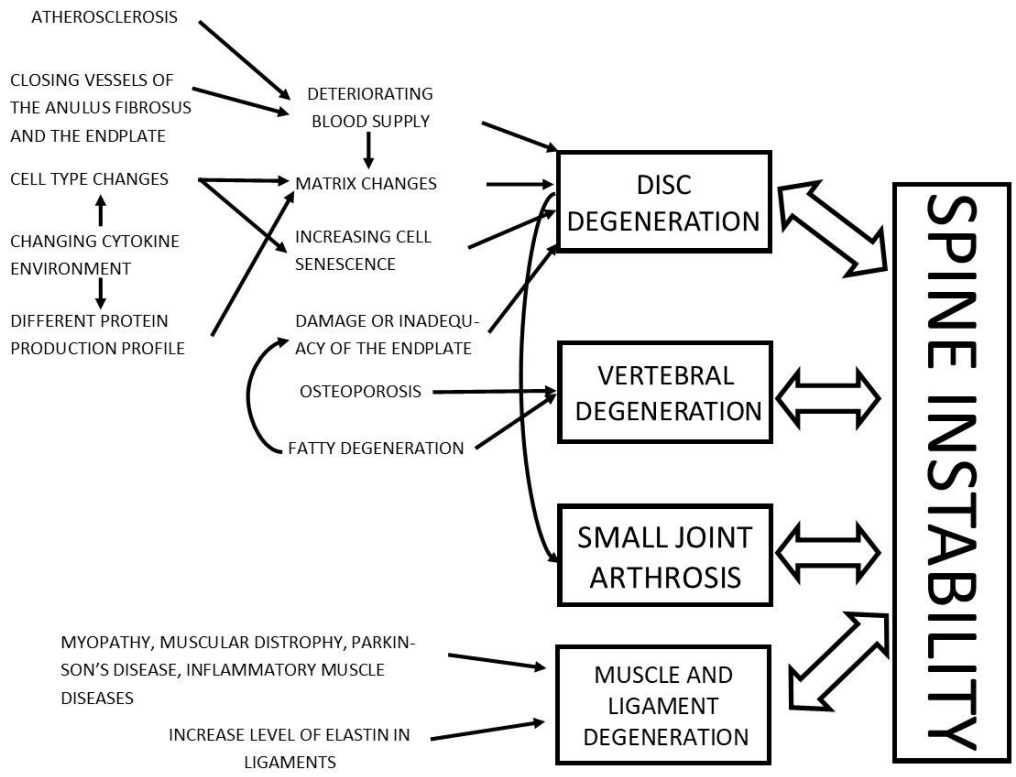

Figure 2: Influencing factors of lumbar degeneration

Lumbar degeneration is a complex, multifactorial process that affects all the structures of the spine (disc, bone, joint, and muscle-ligament system). The degeneration of these structures leads to spine instability, which further enhances the degeneration. (author's own illustration)

1.2.3.1 Intervertebral disc

Disc degeneration is a complex process that is the result of changes in the extracellular matrix (ECM) at the cell and tissue levels. The disc can be divided into two parts: an inner nucleus pulposus (NP) and an external anulus fibrosus (AF) (Figure 3). The consistency of the inner part is gelatinous because of the elastic matrix and high proteoglycan and water content, while the outer part is made up of denser collagen with high tensile strength. The right ratio of collagen-proteoglycan-water is essential for proper function. The NP must resist the high hydrostatic pressure and distribute it to the AF, and the high tensile strength collagens of the anulus fibrosus have to stand against the traction force in the circumference caused by hydrostatic pressure; otherwise, rupture will occur.

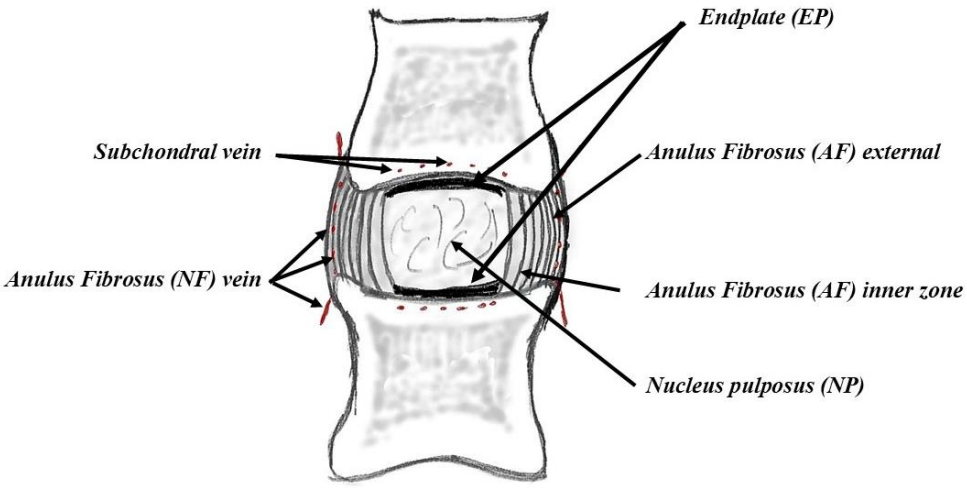

Figure 3: The anatomy and blood supply of the intervertebral disc (author's own illustration)

To repair matrix damage and injuries caused by everyday stress, weightlifting training and heavy physical work, an adequate nutrition supply is required. This nutrition is possible due to the subchondral and anulus fibrosus veins, but the latter veins disappear completely by the age of 40. The only nutrient supply comes from the subchondral vein by diffusion, but the external compression caused by increased load and atherosclerosis

makes this blood supply ineffective.[56] Neovascularisation can be observed, but it is rather a sign of degeneration than of the repair process.[57]

The number of cells also changes during degeneration. First, the initial phase shows a continuous decrease, and then during an exacerbation, the number of cells begins to increase.[57] The type of the cells also changes; at birth notochordal cells are present, which produce a fluid-rich nucleus pulpous matrix, but the number of these cells begins to decrease at an early age, becoming undetectable between 4 and 10 years of age.[58] In place of the notochordal cells, chondritic-type cells appear from the direction of the anulus fibrosus, but because of their different protein synthesis profile and lower matrix production capacity, they cannot replace the function of the original cells. As a result of the altered synthetic activity, the gelatinous consistency of the matrix becomes more cartilage-like, which can also be detected in MRI scans as a decrease in intensity in the T2-weighted images. The inflammatory cytokines such as Tumour Necrosis Factor α (TNF-α), Interleukin 1 (IL1), Interleukin 6 (IL6), Interleukin 8 (IL8) and Interleukin 17 (IL17) increase as well, causing the cells' higher level of catabolic activity (higher level of matrix metalloproteases, MMP), while the synthesis of ECM-forming structural proteins decreases. [57] Furthermore, these cytokines indicate a local immunology response and, with elevated MMP activity, initiate the progress of structural and functional changes. Senescence, which can be defined as a state of irreversible proliferation arrest, is also higher in degenerated cells than in healthy ones.[59] In addition, the reactive oxygen species (ROS) also cause DNA damage which also leads to premature cell ageing. In the disc degeneration process, increased ROS like, nitric oxide (NO) hydroxyl radicals (OH), hydrogen peroxide (H2O2), and superoxide anions ($O2^-$) can be detected. ROS enhance degeneration through different signalling pathways as well (the mitogen-activated protein kinase (MAPK) pathway, nuclear factor-κB (NF-κB) pathway and the lipid pathway (phospholipase, protein kinase C (PKC), and phosphoinositide 3-kinase (PI3K)/Akt pathway)) in addition to their direct DNA damaging effects. Due to the results of these effects, the cell of nucelus pulpusos lose their ability to profilate, making large cell malformation and showing cell cycle stagnation, but these cells also keep their normal function and the ability of protein synthesis.[60]

To summarise, discus degeneration is a complex and multifactorial process. The nutrient supply and the qualitative and quantitative changes in cells are also important factors; other changes such as atherosclerosis, inflammation, reactive oxygen species and environmental factors also impact the degeneration process. According to the study by Rajasekaran et al., there is a "point of no return" from which the disc is unable to restore its original structure and function, so the degeneration process cannot be reversed.[61] Because nutrition is only available from the end plate, it is a crucial structure in the degeneration process, so the EP's mechanical injuries, calcification, and fatty degeneration can also facilitate disc degeneration.

1.2.3.2 Bone

The balance between the resorption and synthesis in bone tissue is important in maintaining proper function. Due to ageing, the balance shifts towards resorption, which leads to osteoporosis. Fatty degeneration, which is the increasing amount of fatty-rich tissue in the bone—especially in the endplate region—is relevant, due to the inhibition of nutrient diffusion from the bone to the disc.[62] As a result of degeneration, the spine becomes unstable and tries to compensate by forming osteophytes to increase the load-bearing surface. The abnormal transformation of the bone structure also affects the small joints and vertebral arches, leading to spinal canal narrowing, as well as the forward slippage of the vertebrae, spondylolisthesis.[63]

1.2.3.3 Small joint

Small joint degeneration is a secondary deviation. Due to disc degeneration, the load on the small joints increases, which leads to erosion of the joint surfaces and the appearance of subchondral sclerosis, cysts, and osteophytes. The degeneration of the joint contributes to the development of low back pain because it is a well-innervated structure.[64] Degenerated small joints can also narrow the spinal canal and can cause spinal canal stenosis.[65]

1.2.3.4 Muscle and ligament system

Any disease that impairs the strength or coordination of muscles contributes to spinal instability and pain. Most commonly, age-related myopathy leads to the weakening of the muscles around the spine, which play a key role in spine stabilization.[63] In addition,

muscle diseases such as Parkinson's disease, inflammatory muscle diseases, and muscular dystrophies also contribute to this process.[65]

The increase of elastin content in ligaments causes a decrease in resistance to tensile forces, which also leads to spinal instability. A good example of this process is the weakening of the anterior and posterior ligaments, increasing the risk of developing disc herniation or bulging.[63]

1.2.4 Examination of patients with low back pain

Generally, if a patient has pain in the lower back region the examination begins with the recording of the patient history, in particular the circumstances, intensity, and frequency of the pain and its nightly appearance. A thorough interrogation will help reveal the pathological process behind the pain. Questionnaires are useful and widely used to record the disability and pain of patients with low back pain. There are several questionnaires, like the Oswestry Disability Questionnaire, the Quebec Back Pain Disability Scale, the Roland-Morris Disability Questionnaire (RMDQ) (Supplement 2), the Waddell Disability Index, and the physical health scales of the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36).[66]

In general clinical practise, this was followed by a physical examination, in which our goal was to localise the pain and isolate the musculoskeletal, neurological, and other internal organ causes. A complete neurological examination can additionally help distinguish radical pain and spinal canal stenosis[67] and help to ensure that the imaging test is carried out optimally.

Because low back pain is most often caused by lumbar degeneration third step is to perform an imaging study, which can be a traditional X-ray examination, although its diagnostic value is considerably limited compared to modern Computer Tomography and Magnetic Resonance Imaging scans, which have consequently become more widespread.

1.2.5 Imaging modalities

1.2.5.1 X-ray

Conventional X-ray is an inexpensive and easily accessible imaging modality which is mainly used to detect bone abnormalities like spondylolisthesis, fractures, the spaciousness of the intervertebral foramen, the sclerosis of the endplate, and signs of tumour infiltration. X-ray is a summation imaging method, so its diagnostic value is limited and further examination is necessary. (Figure 4-5)

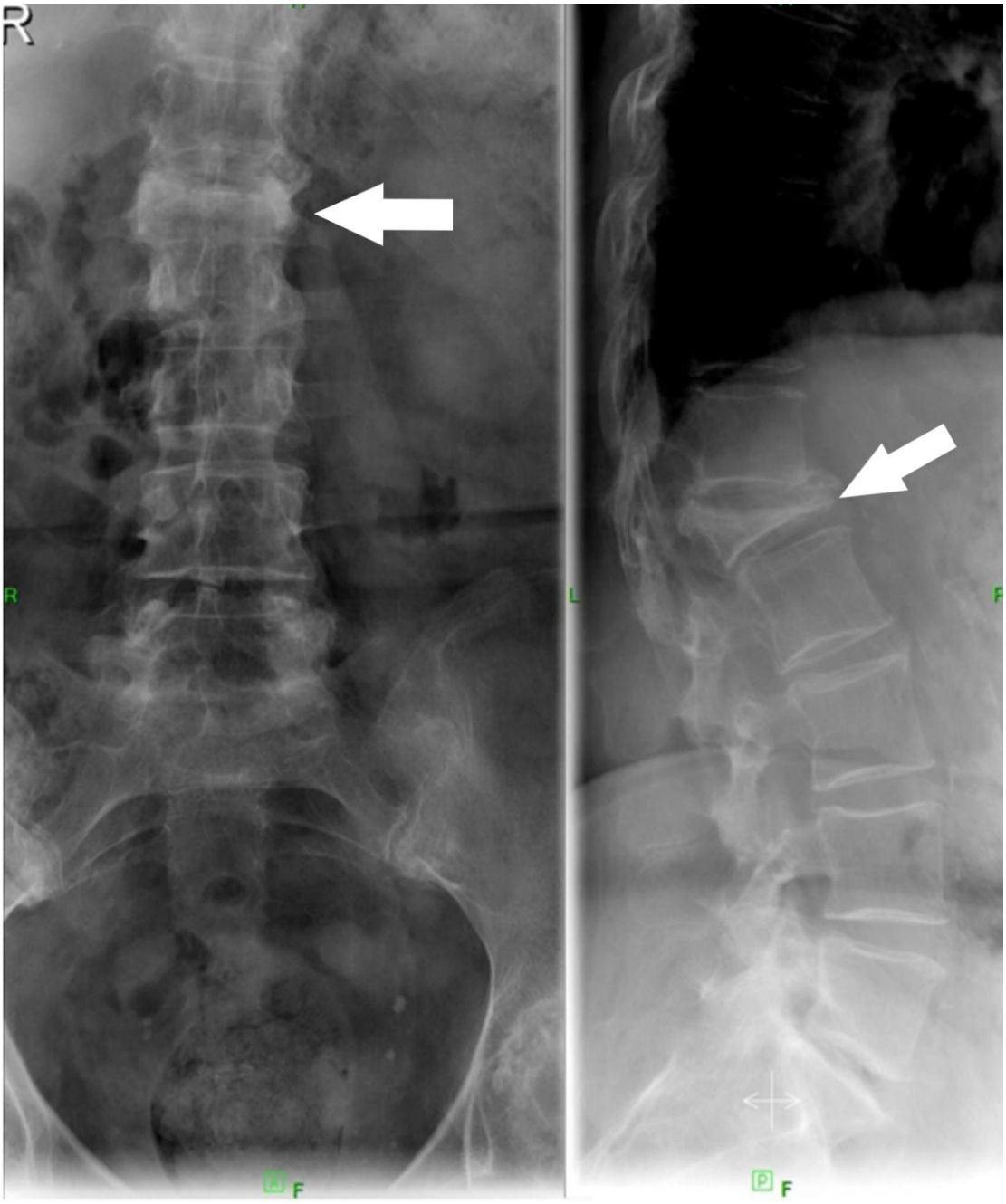

Figure 4: Antero-posterior and lateral projection of the lumbar spine in radiograph.

The bone structure can be easily assessed. The 1st lumbar vertebra body is explicitly wedge-shaped compressed (arrow), most of the endplates have sclerotically degenerated, and the small joints have also degenerated. (Source: Semmelweis University, Medical Imaging Centre)

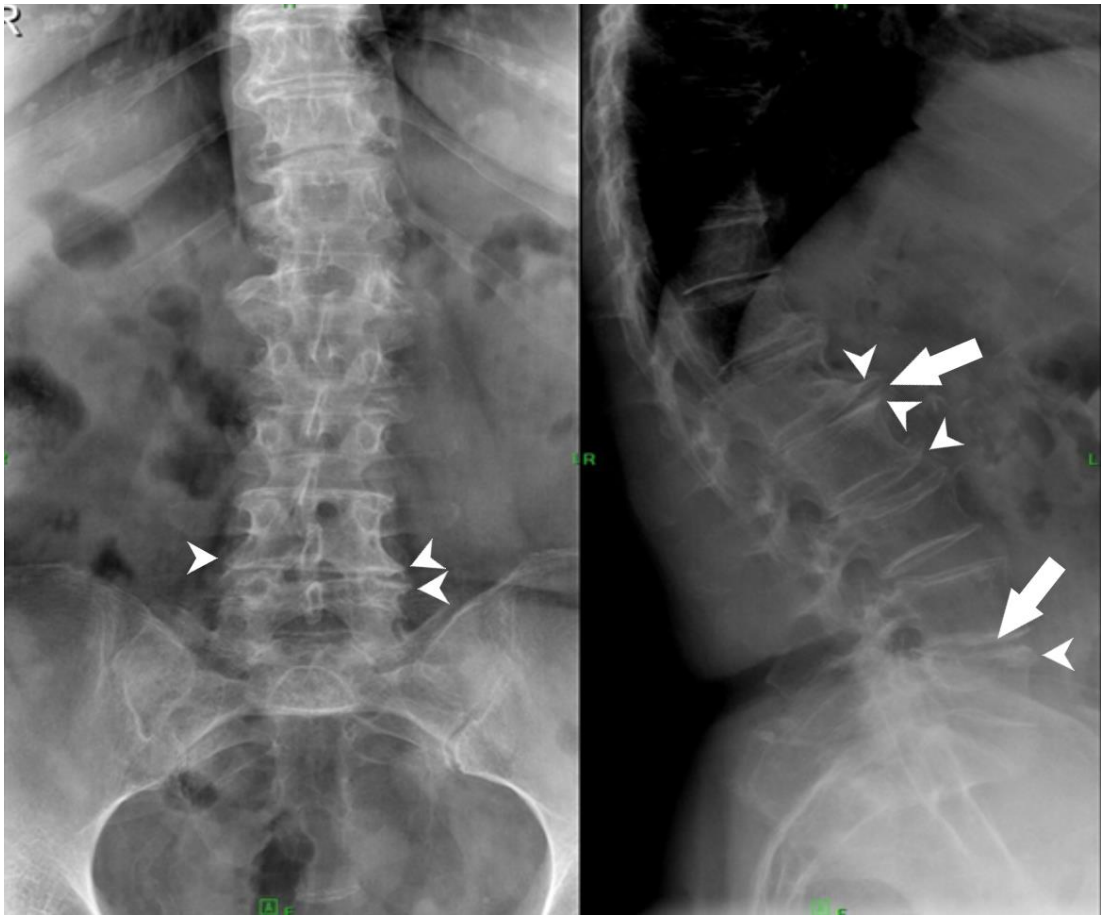

Figure 5: Antero-posterior and lateral projection of the lumbar spine in radiograph.

In these images, there are signs of spondylosis and spondyarthoris in the vertebral bone. The 1st-2nd lumbar and 4th-5th lumbar disc spaces are narrowed (arrows), which can be referred to as degeneration of the disc. Also, the spondylophytes (arrow heads) in the anterior and lateral margins of the vertebral body can also be noticed. (Source: Semmelweis University, Medical Imaging Centre)

1.2.5.2 Computer Tomography

Computer Tomography (CT) gives a three-dimensional view of the spine, and the morphology of bones can be assessed easily in the images. In addition to those abnormalities which can be assessed on the radiograph, the spaciousness of the intervertebral foramen and spinal canal, the internal bone structure of the vertebrae, the presence of osteophytes and other bone structural changes like tumours or metastasis can also be seen. Its limitation is the poor differentiation of soft tissues and the exposure of high levels of ionizing radiation during the examination. (Figure 6-7)

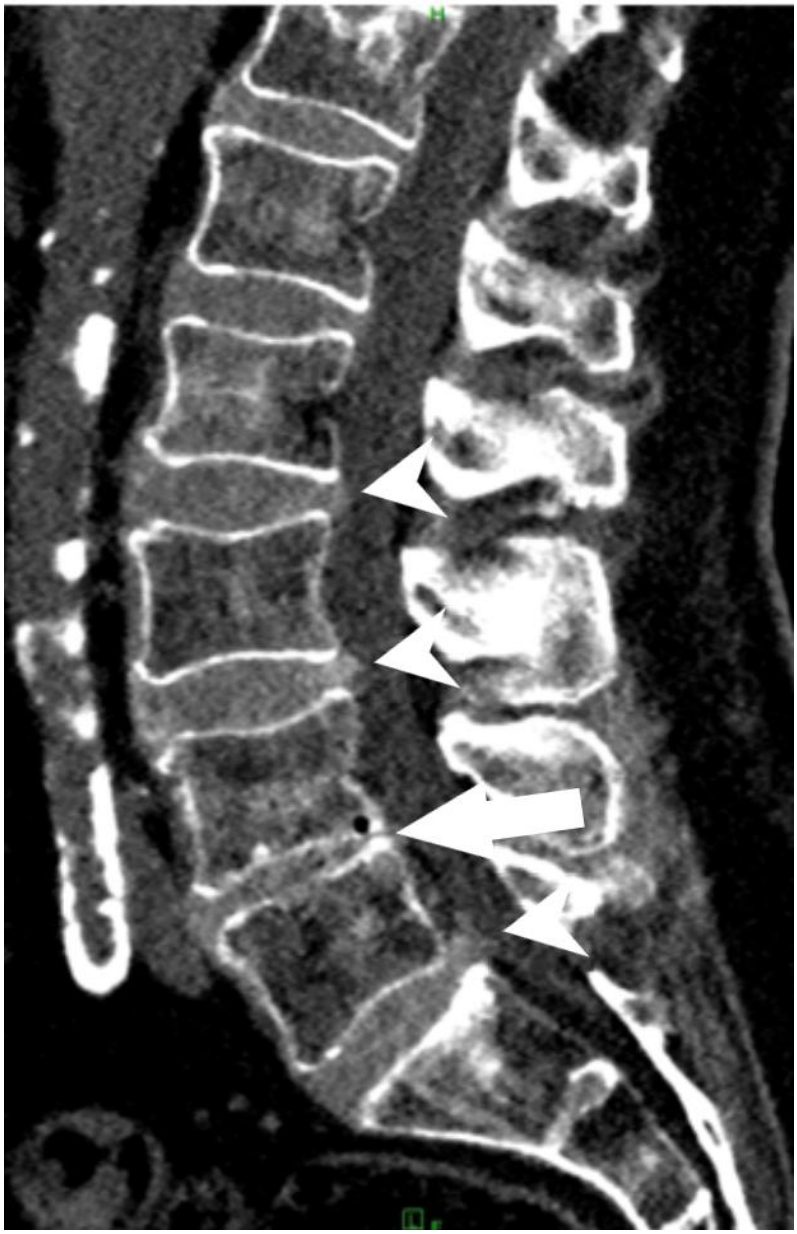

Figure 6: Sagittal view of the lumbar spine region with soft tissue window (window width: 250 HU window level: 50 HU) on CT

The lumbar intervertebral spaces 4-5 are narrowed (arrow)and the 2^{nt}-3rd lumbar, 3nd-4th lumbar, and 5th lumbar- 1st sacral discs seem to be bulged backward (arrow heads). As seen, the soft tissue resolution is limited. (Source: Semmelweis University, Medical Imaging Centre)

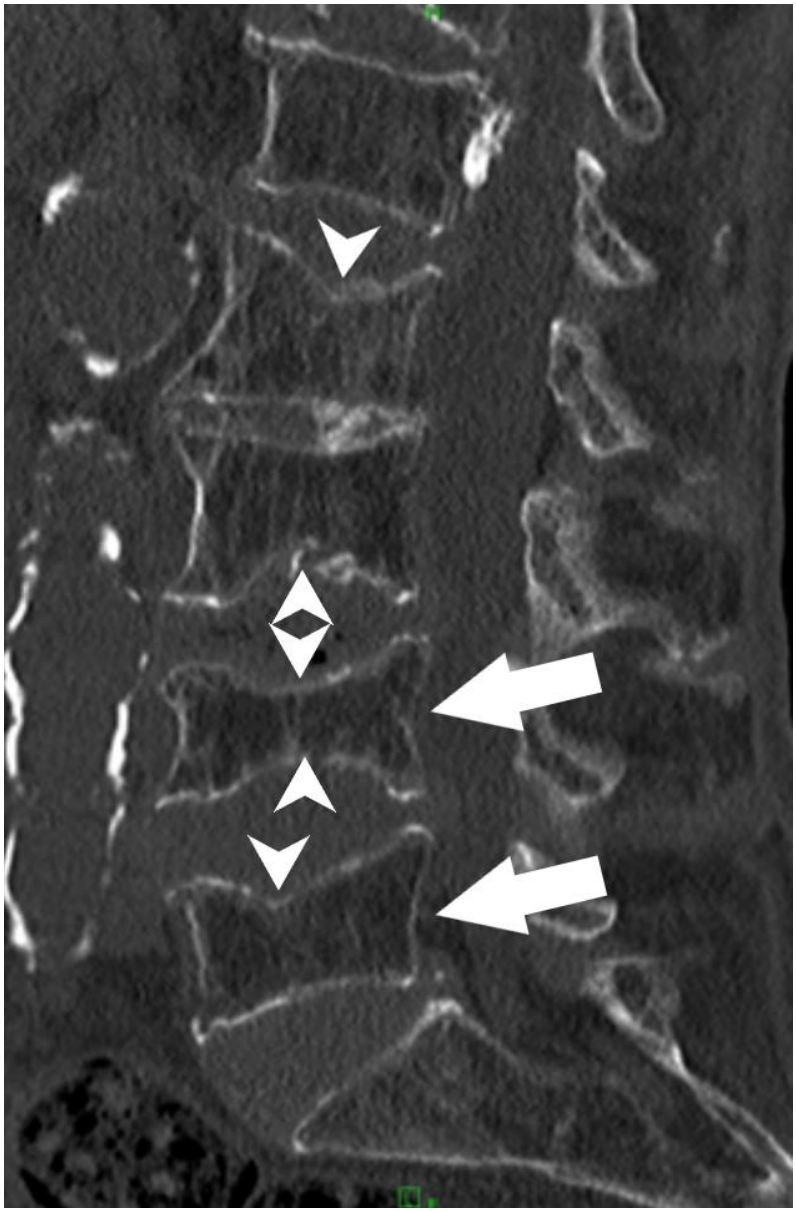

Figure 7: Sagittal view of the lumbar spine region with bone window(window width: 1800 HU window level: 400 HU).

There is explicit spondylosis, the 4th and 5th lumbar vertebra bodies are flat (arrows), and most of the endplate is degenerated (arrow heads). As can be seen, the bone structure can be easily assessed. (Source: Semmelweis University, Medical Imaging Centre)

1.2.5.3 Magnetic Resonance Imaging

MRI examination is the most suitable method for examining soft tissues and the spinal cord. During the examination of the lumbar spine, it is recommended to take T1 and T2 weighted sagittal, and T2 weighted coronal and axial shots routinely. In the case of processes associated with oedema, such as degeneration or tumour lesions, it is also worth taking axial or coronal STIR (short tau inversion recovery) sequences, on which the water content/oedema is easier to assess.

Coronal T2 weighted images clearly show root bags, root exits, oedemas, bleeding, and ligament tears. T1 scans help to judge general anatomy, and bleeding and bone metastases are also clearly depicted. STIR sequences allow superior visualisation of degeneration-induced oedema and bone metastases. Depending on the clinical state in the case of low back pain, gadolinium contrast is often used. With contrast enhancement, many abnormalities like tumours, post-operative status (for example, distinguishing recurrent disc herniation from epidural scarring) and inflammation can be easily assessed. [66]

1.2.6 MR abnormalities

Low back pain is associated with MRI changes, accordingly, the most common abnormalities in lumbar spine MRI will be discussed in the following section.

1.2.6.1 Vertebral lesions

1.2.6.1.1 Fatty degeneration of the vertebrae

During the ageing process, adipose tissue accumulates in the bone marrow or replaces the normal bone tissue which can be detected as an increase in signal intensity in T1 weighted images. (Figure 8)

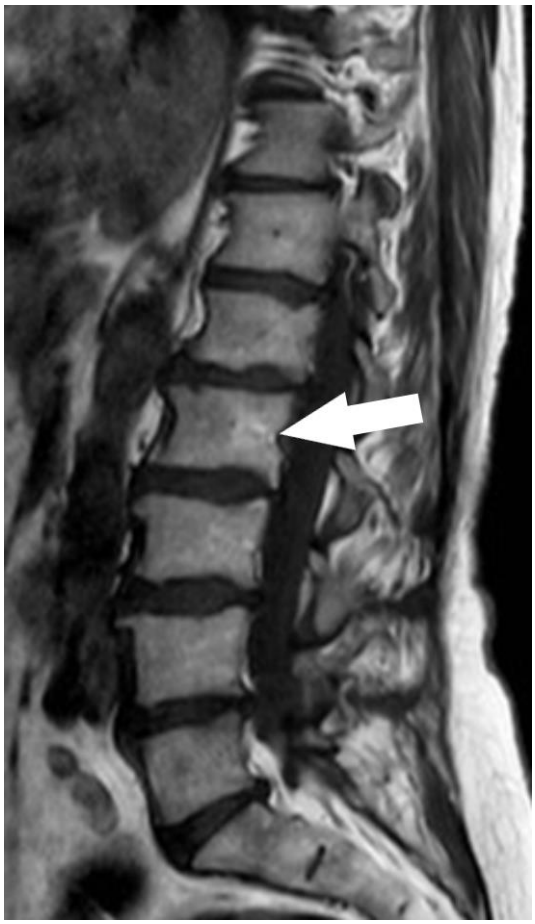

Figure 8: T1 weighted sagittal MRI scan of lumbar spine.

An increase in signal intensity observed in the stock of vertebrae is a sign of fatty degeneration. (Source: Semmelweis University, Medical Imaging Centre)

1.2.6.1.2 Spondylophytes

The vertebra responds to spine instability with abnormal bone growth. Osteophytes are one of the most common pathological pointed bone growths; they appear on the surface of the vertebral body in contact with the intervertebral discs. (Figure 9) Their appearance is a sign of degeneration; in addition, if the osteophytes grow backwards near the spinal canal, they can cause spinal canal stenosis.

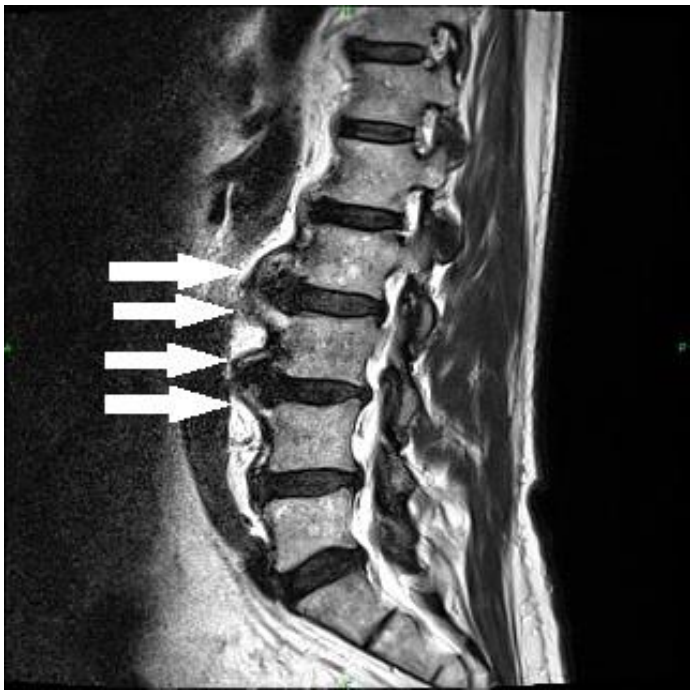

Figure 9: *Spondylophytes of the lumbar spine.*

On this T2 weighted sagittal MR image of the lumbar spine region, spondylopyhtes (arrows) beside the degenerated 2nd and 4th lumbar discs—can be observed. (Source: Semmelweis University, Medical Imaging Centre)

1.2.6.1.3 Spondylolisthesis

As a result of the degeneration of the intervertebral disc and small joints, the vertebrae can slip out of their anatomical positions. (Figure 10) The position of the vertebra is always relative to the vertebra located below, so in anterolisthesis the vertebrae move forward, in the retro/posterolisthesis they move backwards, and in laterolisthesis the vertebrae move right or left.[63] Other causes like traumatic events, tumours, osteoporosis, or malformation can also be in the background of the pathological localisation of the vertebra.

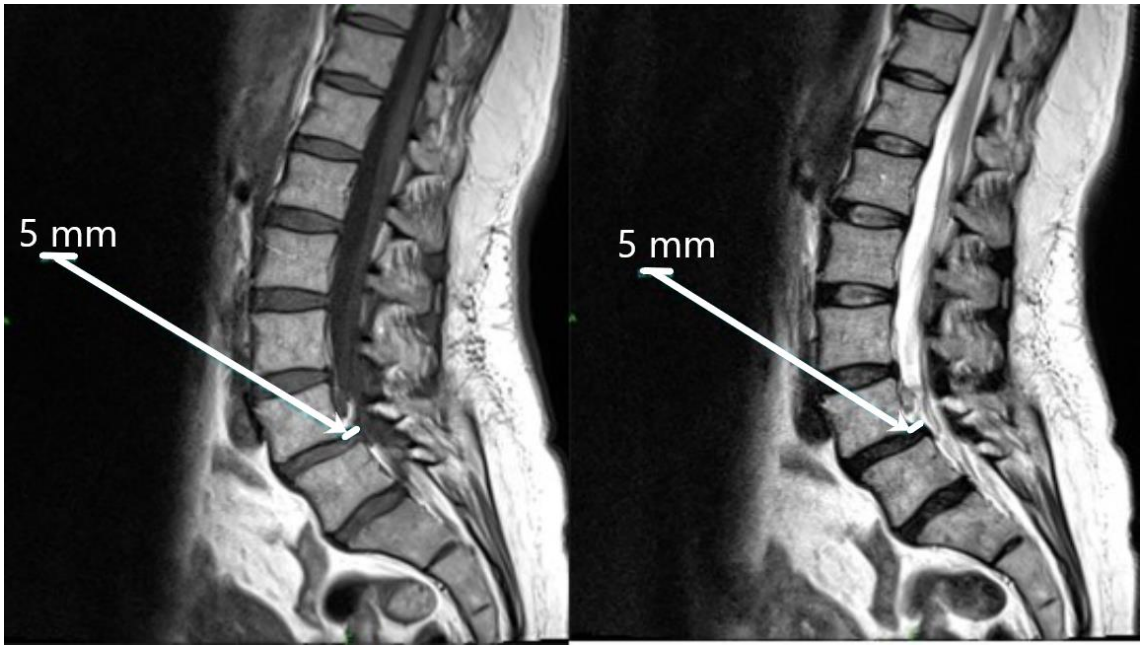

Figure 10 : Spondylolisthesis on MRI.

On the left is a T1-weighted sagittal image, and on the right, a T2-weighted sagittal image of the lumbar spine region. In both images you can see that the 4th lumbar vertebra is located 5 mm anteriorly to the 5th lumbar vertebra, indicating anterolisthesis. (Source: Semmelweis University, Medical Imaging Centre)

1.2.6.1.4 Spinal canal stenosis

Normally, the sagittal diameter of the spinal canal on the lumbar section is 16-18mm. If this diameter decreases, spinal canal stenosis exists. In most cases, stenosis has symptoms like low back pain, numbness, stiffness and loss of bowel and bladder control. It is most often caused by arthritis, but herniated discs, injuries, tumours, Paget's disease, and thickened ligaments can also cause stenosis.[68] (Figure 11)

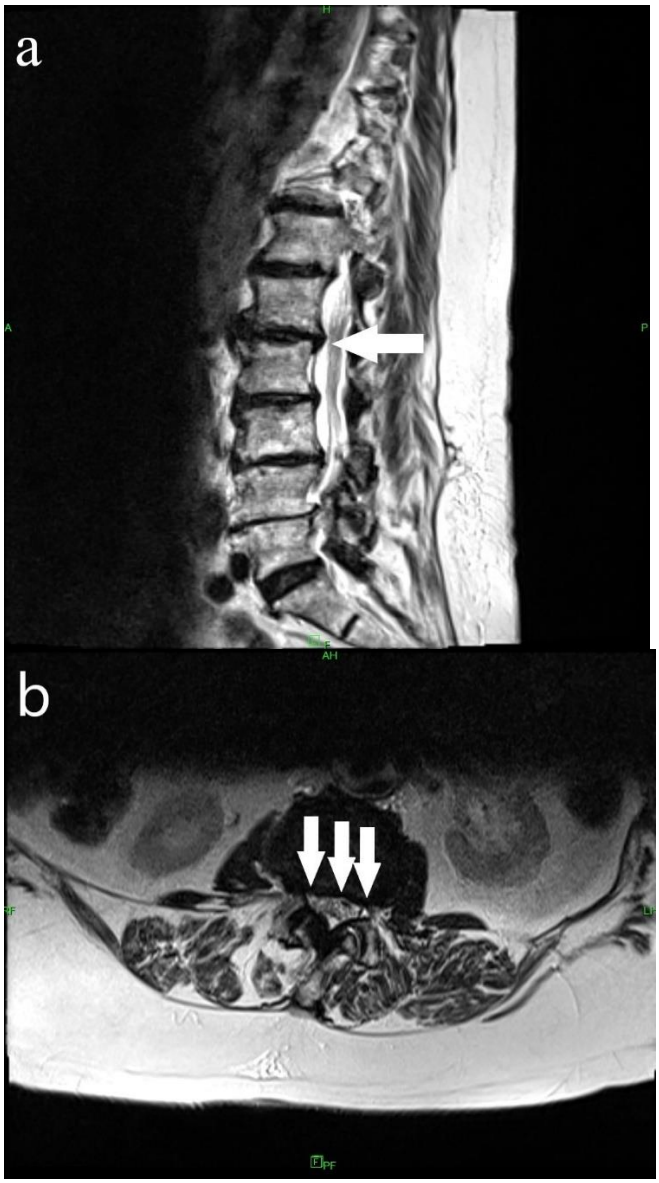

Figure 11: Spinal canal stenosis:

a, T2 weighted sagittal MR image of the lumbal spine: the 1st lumbal discus forms an impression on the dural sac and narrows the spinal canal (arrow); b, an T2 weighted axial image of the 1tsth lumbal discus. In these images, you can observe the bulging disc make a great impression, significantly narrow the spinal canal and compress the spinal cord, (Source: Semmelweis University, Medical Imaging Centre)

1.2.6.1.5 Intervertebral foramen stenosis

The intervertebral foramen is a bone aperture between two vertebrae where the spinal nerve leaves the spinal canal. The lateral recess is the region of the lumbar canal that is bordered anteriorly by the vertebral body, endplate margin, and disk margin; posteriorly by the superior articular facet and ligamentum flavum; and laterally by the pedicle.[69] In the majority of cases, the bone growth of the degeneration of small joints or protrusion of the intervertebral disc is behind the narrowing where the exiting nerve is compressed, causing radiculopathy. (Figure 12)

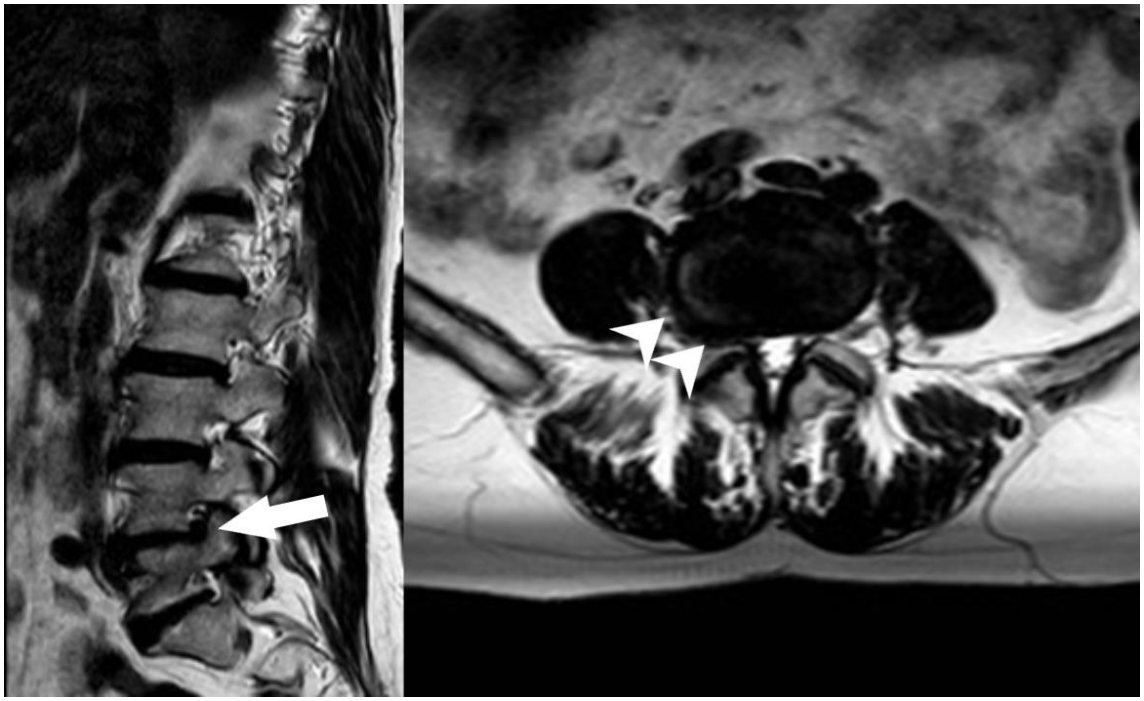

Figure 12: Intervertebral foramen stenosis.

On the left side: T2 weighted sagittal MR image of the lumbar spine, on the right axial T2 weighted image of 5th lumbar disc. As you can notice the asymmetric bulging of the 5th lumbar disc narrows the spinal canal (arrow) and on the right side the intervertebral foramen and lateral recess (arrow heads). (Source: Semmelweis University, Medical Imaging Centre)

1.2.6.2 Intervertebral disc abnormalities

1.2.6.2.1 Disc height reduction

One of the easiest abnormalities used in testing for disc degeneration is the examination of intervertebral disc height. We cannot determine objectively the normal disc height range, so we compare the measured heights to each other. In a physiological case, the height increases in the direction of the craniocaudal, so if the height of a discus is the same or less than the one located above it, it is considered narrowed and degenerate.[70] (Figure 13)

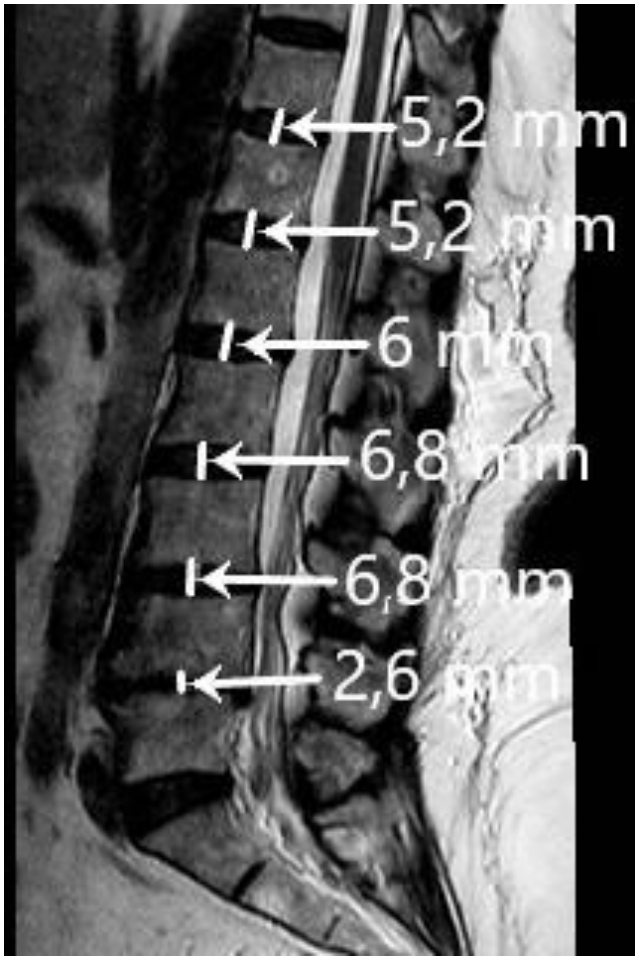

Figure 13: Disc height reduction

T2 weighted sagittal MRI scan: The height value of each disc can be seen in the footage. The 3rd-4th lumbar disc is only 6,8 mm, and the 4th-5th lumbar disc is only 2.8 mm, which means its height is decreased. (*Source: Semmelweis University, Medical Imaging Centre)*

1.2.6.2.2 Disc dehydration

The nucleus pulposus and the inner zone of anulus fibrosus have a higher water content due to their proteoglycan content, so T2 weighted images show a higher signal intensity. Liquor, which has a similar signal intensity, helps to assess the normal water content of the disc. During ageing, the proteoglycan and water content of the disc decrease, so a decrease in signal intensity is visible in T2 weighted images (Figure 14). In many cases, this signal intensity decrease is also accompanied by a height reduction of the disc.[71]

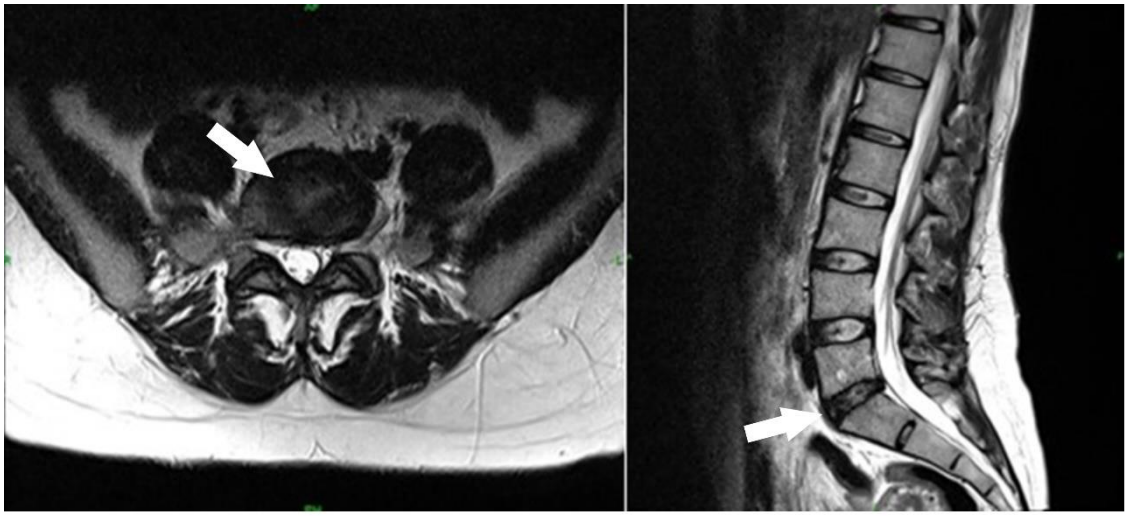

Figure 14: Disc dehydration of the 4^{th}–5^{th} lumbar disc.

On the left, the T2 weighted axial image of the 4^{th}–5^{th} lumbar disc and on the right, T2 weighted sagittal MR image of the lumbar region can we have seen. In both images the 4^{th}–5^{th} lumbar disc shows lower signal intensity on T2 weighted images, indicating that the disc is dehydrated (arrow). (Source: Semmelweis University, Medical Imaging Centre)

1.2.6.2.3 Disc bulging

During the degeneration process, the external part of the anulus fibrosus becomes weaker, so the disc starts flattening. If this process affects more than a quadrant section (i.e., more than 25% [90°] of the circumference), it is called asymmetric bulging; if it influences the whole circumference, it is called symmetric or circumferential bulging. (Figures 15 and 16). The change in disc shape is caused by altered protein synthesis, which is not necessarily accompanied by water content loss in the disc or a consequent signal intensity reduction on T2-weighted images.[71]

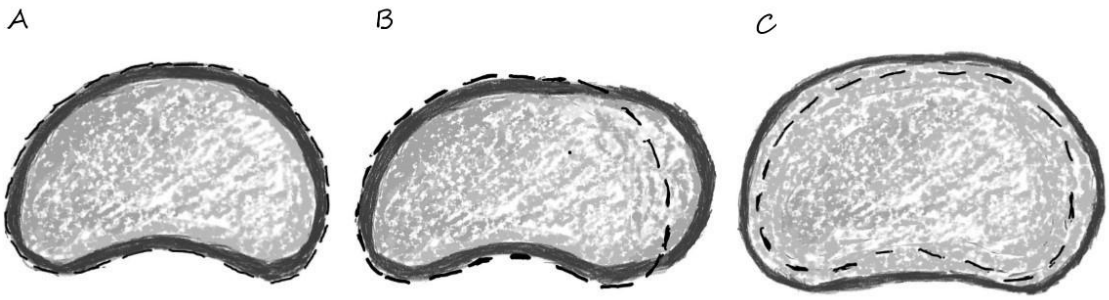

Figure 15: Schematic drawing of discus bulging: A–normal discus, B–asymmetrical bulging, C–symmetrical bulging (author's own illustration)

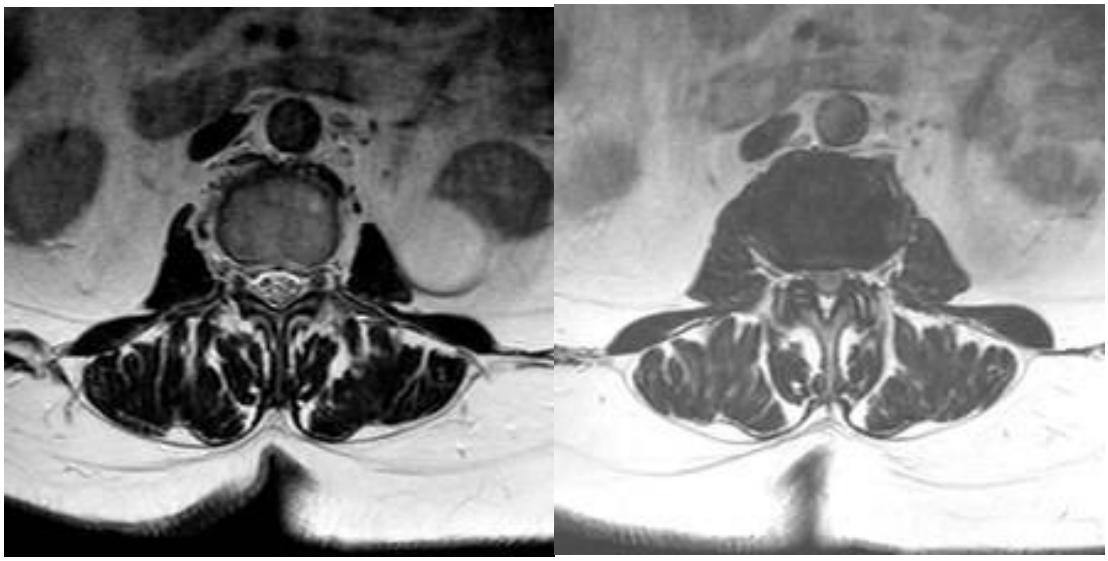

Figure 16: Disc buging

On the left, T2 weighted axial image of the 4th vertebra; on the right, T1 weighted axial image of the 4th-5th lumbar discus. The diameter of the discus is visibly larger than the diameter of the vertebra, a sign of lumbar disc bulging. (Source: Semmelweis University, Medical Imaging Centre)

1.2.6.2.3.1 Disc herniation

Disc herniation means that the disc leaves the boundary of the edge of the vertebrae and the lesion affects a quadrant section of the circumference (less than 25%). Different forms of herniation are distinguished:

- The protrusion is when the disc spills out with a wide base and the protruded part remains at the level of the disc.[72] (Figures 17 and 18) Protrusion does not affect the outer part of the anulus fibrosus, which remains intact. Five different forms are distinguished in terms of anatomical localisation: anterior, lateral, foraminal (intervertebral foramen), subarticular, and central.

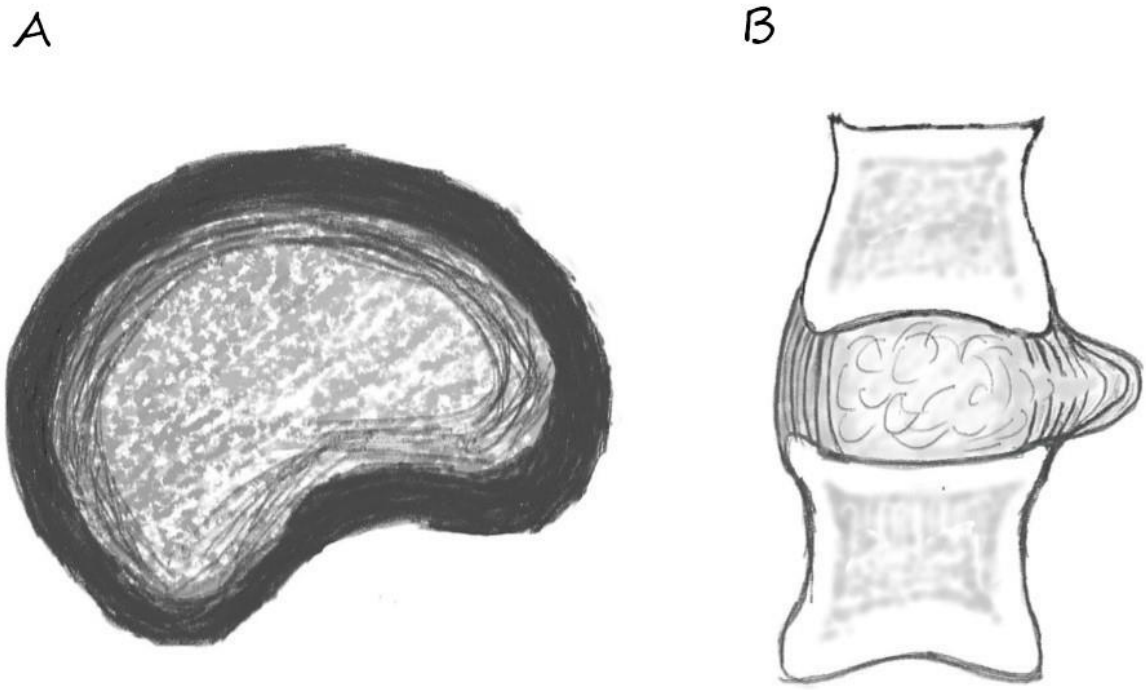

Figure 17: Schematic diagram of disc protrusion.

A–axial image of protrusion; B–sagittal image of the protrusion (author's own illustration)

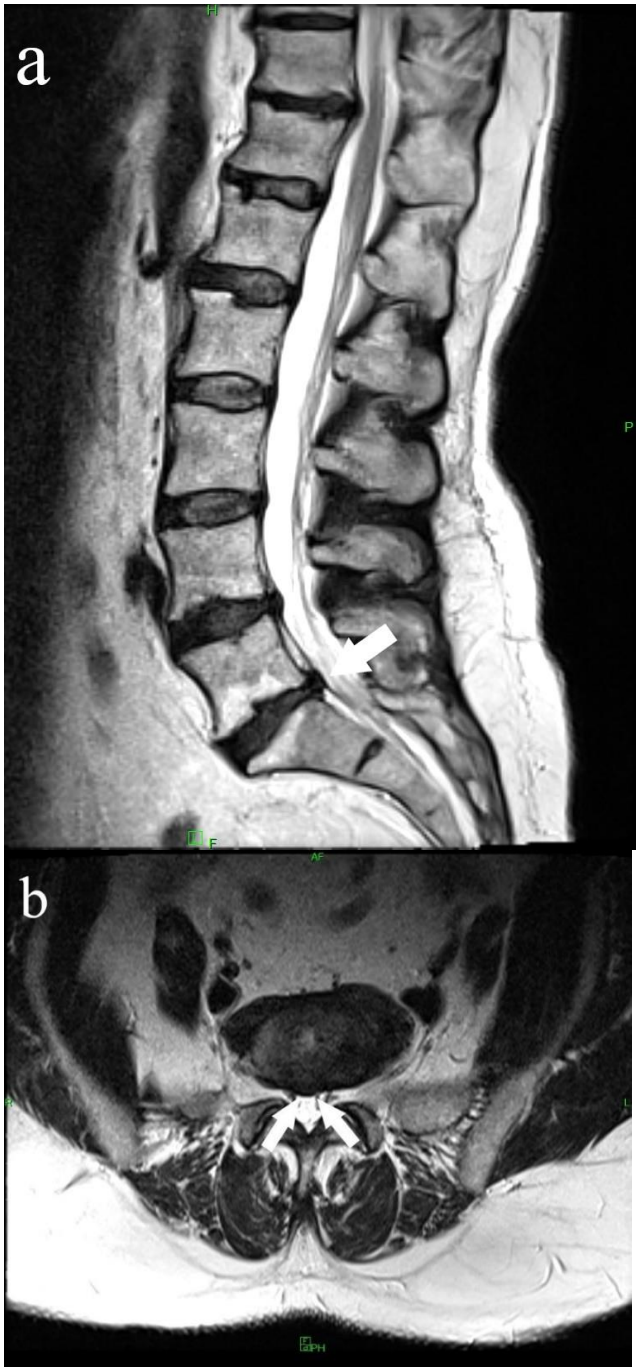

Figure 18: Disc protrusion

a, T2 weighted sagittal image of the lumbal spine; b, T2 weighted axial image 5th lumbar disc. As you can notice in both images the 5th lumbar disc has a small central protrusion (arrows) (Source: Semmelweis University, Medical Imaging Centre)

- Extrusion is a type of disc herniation where the bulge has a tighter basis (like a neck) and a broader dome. (Figure 19) In many cases, annular tear phenomena or annular fissures can also be observed in the inner part of the anulus fibrosus where the nucleus pulposus has herniated.

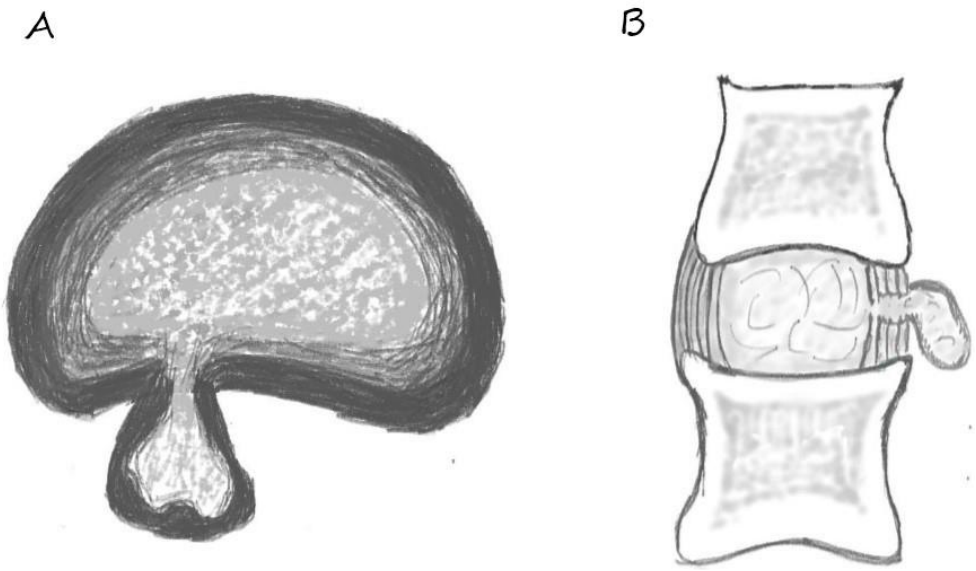

Figure 19: Schematic diagram of disc extrusion.

A–Axial view, B–sagittal view. The bulge has a narrow neck and broader dome. The bulge has a connection with the disc. (author's own illustration)

- Sequestration herniation is when the protruding part can separate from the disc and migrate like a free segment from the level of the disc. (Figure 20)

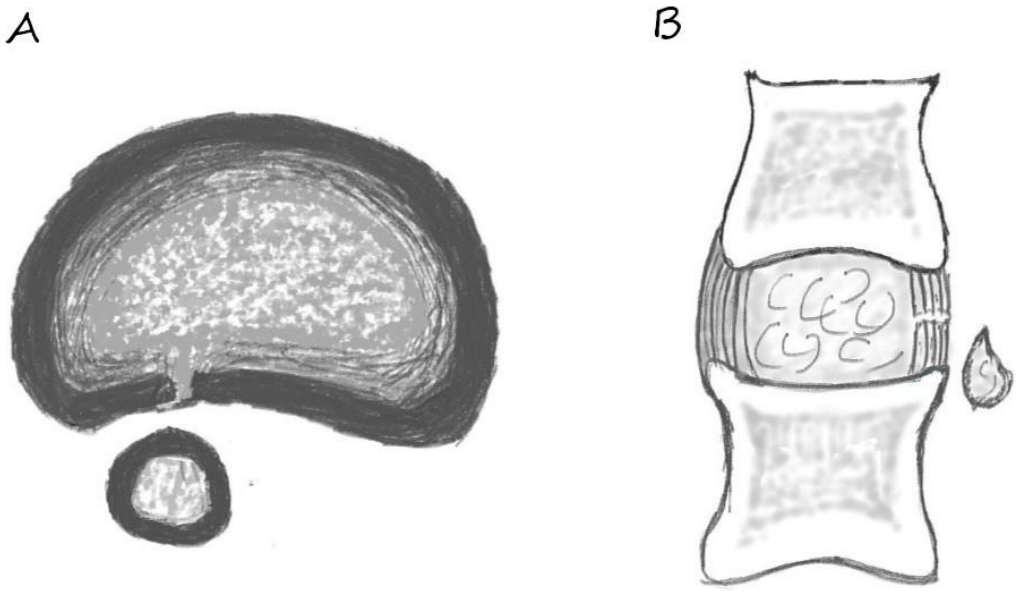

Figure 20: Schematic diagram of disc sequestration

A–Axial view, B–sagittal view: There is no connection between the discus part that has protruded and then torn off and the discus that remains in its original place. (author's own illustration)

- Schmorl hernia: The free segment can migrate toward the endplate, break it, and become inserted in the vertebral bone tissue. This abnormality is called a Schmorl hernia, which can also be defined as an endplate break with a depression of subchondral bone.[72] (Figure 21)

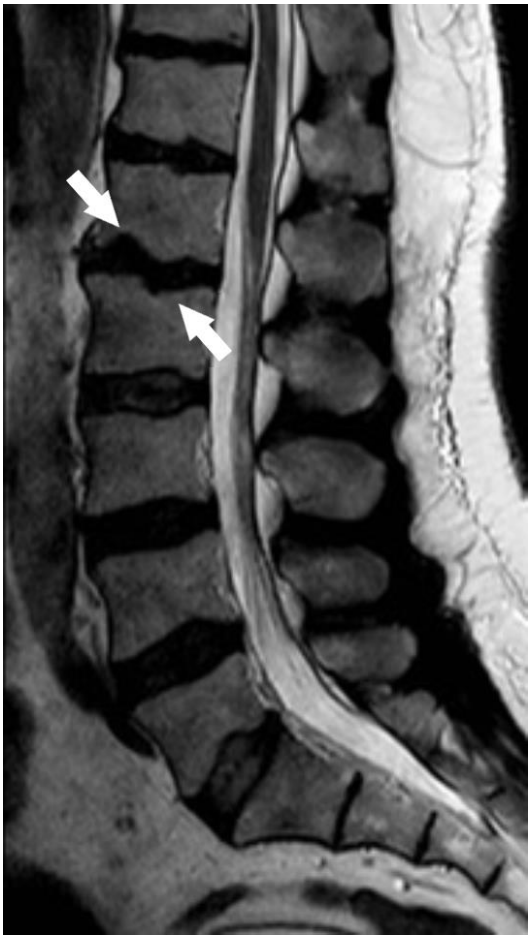

Figure 21: Schmorl hernia

T2 weighted sagittal MR image: A Schmorl hernia (arrows) is depicted on the 1st-2nd lumbar discus. (Source: Semmelweis University, Medical Imaging Centre)

A herniated discus can be classified into contained and uncontained groups. In the contained type, the bulged part is covered by anulus fibrosus or ligaments.[73]

1.2.6.2.4 High Intensity Zone (HIZ)

An HIZ appears in T2 weighted images as an area in the anulus fibrosus (usually at the back side) that has higher signal intensity than the nucleus pulposus. In many cases, it can correspond to the phenomenon of anulus fissure or an anulus fibrosus rupture of degenerative origin.[72] In the background, vascularized granulation tissue appears in the anulus fibrosus due to the inflammatory process, which has a higher signal intensity in T2-weighted MRI images. (Figure 22)

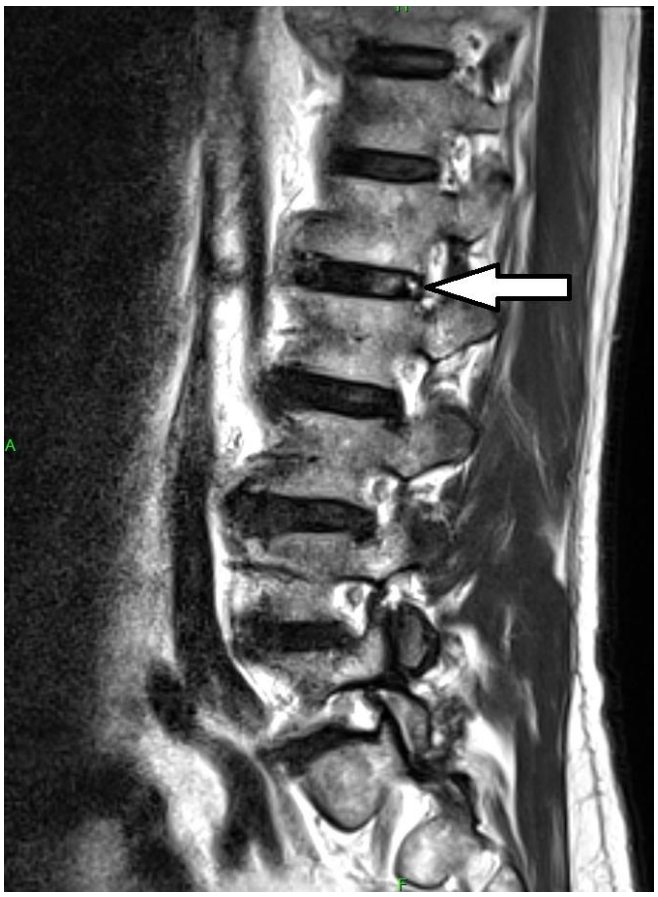

Figure 22: High Intensity Zone

T2 weighted sagittal image of the lumbar spine region. There is a small, point-like high signal intensity area in the outer part of the 2nd-3rd lumbar disc, which is called the High Intensity Zone. This lesion suggests that the anulus fibrosus is ruptured. (Source: Semmelweis University, Medical Imaging Centre)

1.2.6.2.5 Pfirrmann score

The Pfirrmann score characterises the condition of the disc, classifying the disc into one of five types based on T2 weighted MRI images.(74,75) For a detailed description of the points system, see Table 1.

Table 1: The criteria of the Pfirrmann score

Grade	Height of the intervertebral disc	Structure	Distinction of nucleus and anulus	Signal Intensity
I	Normal	Bright white, Homogenous	Clear	Hyperintense or isointense to cerebrospinal fluid
II	Normal	Inhomogeneous with or without horizontal bands	Clear	Hyperintense or isointense to cerebrospinal fluid
III	Normal or slightly decreased	Inhomogeneous, grey	Unclear	Intermediate to cerebrospinal fluid
IV	Normal or moderately decreased	Inhomogeneous, grey to black	Lost	Intermediate to hypointense to cerebrospinal fluid
V	Collapsed disc space	Inhomogeneous, black	Lost	Hypointense to cerebrospinal fluid

1.2.6.2.6 Total Endplate Score (TEPS)

Total Endplate Score is a scoring system used to assess the level of degeneration of the end plate. The Total Endplate Score is the sum of score points of end plates located below and above the disc. Six types of degeneration levels are determined, so endplate scores range from 1 to 6; it follows that the TEPS is between 2 and 12 points, see Table 2. [76]

Table 2: The criteria of Total Endplate Score

Type	Description	Point
I.	No EP breaks or defects Uniform hypointense band Symmetrically concave Not associated with Modic change	1
II.	Focal thinning of EP No EP breaks No Modic change	2
III.	Focal disc marrow contacts Normal contour of the EP maintained No Modic change	3
IV.	Defect up to 25% of width of EP Typical depression present Modic change usually presented	4
V.	Defect up to 50% of width of EP Typical depression present Modic change usually presented	5
VI.	Completed EP damage Irregularity and sclerosis of EP Modic change usually presented	6

1.3 A common biomarker in OSA and lumbar degeneration: protein klotho

As previously mentioned, OSA may lead to spinal deformities due to vitamin D deficiency and chronic intermitting hypoxia which stimulates osteoclast formation, downregulates osteogenic differentiations, and causes early mobilisations of mesenchymal stem cells, of which the last is an important factor in the differentiation of osteoblast and endothelial progenitor cells.

Chronic intermitting hypoxia (CIH) is a frequently occurring phenomenon in OSA which increases the reactive oxygen species and the pro-inflammatory mediators.[49] In OSA, the elements of systematic inflammation are poorly described—especially the immune regulators. A possible connection between the two diseases at the molecular level is the klotho protein, which is described as an anti-ageing protein, as proven by klotho gene-deficient mice having premature death, early ageing, and multiorgan damage. The klotho family has three members (a, b, and c klotho) and is synthesized by many organs like the distal convoluted tubules of the kidney, parathyroid gland, ovary, testis, and placenta.[77] The protein has soluble and membrane-bound forms.[78] The function of the klotho proteins is an anti-inflammatory, anti-oxidative effect and promotes the expression of endothelial nitric oxide synthase (eNOS). Systematic inflammation reduces the klotho protein level[79] and, as previously mentioned, OSA decreases the level of vitamin D[80], which is also a klotho protein activator. Morbid obesity is also associated with lower klotho levels and is one of the main risk factors in OSA. Pákó et al. have shown that chronic intermitting hypoxia reduces the klotho protein level in OSA patients, and the decreased level of klotho protein may play a role in enhanced systematic inflammation.[77] Furthermore, in connection with lumbar degeneration the decreased level of klotho protein is also associated with pathophysiological bone loss in ageing[81], spinal disease,[82] osteocalcin levels,[81] and bone mineral density.[82] Inflammatory factors like interleukin-1β (IL-1β), tumour necrosis factor α , and prostaglandin E2 (PGE2), promote the intervertebral disc's degeneration, and in the degenerated disc a lower klotho protein level was detected. The klotho protein inhibits the inflammatory pathways, so it may have a role in the pathological process of lumbar degeneration as well.[83]

In conclusion, a molecule which could be a potential link between OSA and discopathy is the anti-inflammatory klotho protein, whose levels were found to decrease both in

plasma samples of patients with OSA[77] and in the nucleus pulposus specimens in disc degeneration.[83]

2 Objectives

As shown in the introduction, OSA and LBP are conditions that affect a wide range of people in society and have a major negative impact on patients' quality of life. In many cases, OSA is discovered late after the onset of more severe symptoms, so it is important to research the factors involved in its development. Family[20] and twin studies(23,24) performed on the heritability of OSA suggest that it is a hereditary disease, but these studies were questionnaire-based and have limited ability to detect heritability. Our research group aimed to investigate the factors influencing OSA in twins using an objective polysomnography method. We aimed to confirm the heritability hypothesised by previous studies and to investigate the significance of other influencing factors such as body weight, hypertension, etc. We hypothesise that OSA shows a significant heritability predisposing to the development of the disease, which calls for a screening of relatives of patients with OSA. The suspected heritability of OSA is also a significant factor in the prevention of the development of the disease, as it is particularly important to raise awareness of the importance of avoiding environmental factors that play a role in the pathogenesis of OSA, such as obesity, smoking, etc.

Patients with OSA include a high proportion of people with chronic low back pain as well.[50] Previous studies have found a link between OSA and degenerative changes in the cervical spine[43], but interestingly the lumbar region has not been previously investigated. Low back pain is most often caused by degenerative processes of the lumbar spine, but rarely other causes are also possible. Previous studies have suggested that OSA promotes lumbar degeneration through chronic intermitting hypoxia[46] and impaired vitamin D metabolism[48]; in addition, the role of the anti-inflammatory klotho protein has been implicated as a common pathogenetic factor.[83] We hypothesized that OSA promotes the development of lumbar degeneration, so our participants underwent a lumbar MRI scan to look for abnormalities of degeneration and to assess the degree of pain localised to the lumbar region. As long as there is some kind of association between the two diseases, attention should be drawn to the low back pain of patients with OSA.

3 Methods

3.1 The Sleep Study

Seventy-one Hungarian twin pairs (48 monozygotic, MZ and 23 dizygotic, DZ) from the Hungarian Twin Registry attended the sleep study. Pregnant subjects, patients with uncontrolled chronic cardiorespiratory disease (i.e., asthma exacerbation or acute heart failure) and those with an acute respiratory infection within 4 weeks of measurement were excluded. None of the participants was previously diagnosed with OSA or had received any treatment, including positive airway pressure therapy, surgical treatment or a mandibular advancement device. All applicants had Caucasian ethnicity and their zygosity was measured with a standardised questionnaire.[84] Smoking habits were recorded and sorted into three categories: never smoked, former smoker, or active smoker. Pack-years were calculated as Number of pack years = (number of cigarettes smoked per day × number of years smoked)/20. Weight was measured with the OMRON BF500 monitor (Omron Healthcare Ltd., Kyoto, Japan). Body mass index was determined by the weight (kg)/height $(m)^2$.

Sleep studies were performed in the Department of Pulmonology, Semmelweis University. The twin pairs filled out an Epworth Sleepiness Scale (ESS) questionnaire (Supplement 1). Their medical history was taken and blood pressure and heart rate were recorded. Afterwards, either an overnight polysomnography (PSG) using the Somnoscreen Plus Tele PSG (Somnomedics GmbH, Germany) or a cardiorespiratory polygraphy using Somnoscreen RC devices (Somnomedics GmbH) was performed according to the international recommendation.[85] Accordingly, electroencephalography, electrooculography and electromyography, body position, chest and abdominal movements, intranasal pressure, electrocardiography, and oxygen saturation were recorded. On the recommendations of the American Academy of Sleep Medicine (AASM) [86], sleep stages and arousals, movements, and cardiopulmonary events were scored manually, and total sleep time (TST), percentage of sleep time spent with oxygen saturation below 90% (TST90%), minimal oxygen saturation (MinSatO2), arousal index, respiratory arousal index (RespAI), and sleep period time (SPT) were registered, and the sleep efficiency was calculated as TST/SPT. In this study, apnoea was defined as a 90% decrease in nasal airflow lasting for more than 10 s, and hypopnea was defined as at least a 30% airflow decrease lasting for at least 10 s, which related to a $\geq 3\%$

oxygen desaturation or an arousal. The presence and severity of OSA were determined by calculating the apnoea-hypopnea index , the respiratory disturbance index, and the oxygen desaturation index . Following the PSG or the cardiorespiratory polygraphy in the morning, blood pressure and heart rate were recorded.[87] The following day fasting blood was taken for klotho which was measured with a commercially available enzyme-linked immunosorbent assay as described previously.[77]

The research was conducted in accordance with the Declaration of Helsinki. The local ethical committee (Semmelweis University TUKEB 30/2014, amended on 10 October 2016 and 7 December 2018) approved the study, and all subjects gave informed consent prior to study entry.

3.2 Study on the association of OSA with lumbar spine MR abnormalities

All subjects from the previous study were invited to a lumbar MRI at the Medical Imaging Centre, Semmelweis University or Borsod-Abaúj-Zemplén County Hospital. As previously mentioned, none of the participants had been previously diagnosed with OSA, and none of them had had clinically or MRI-confirmed lumbar spine disease, hence none had received any treatment for these. From the previous sleep study, 56 participants (inpatient polysomnography [n = 39] or cardiorespiratory polygraphy [n = 17]) were further examined in connection with lumbar degeneration. Before the MRI scan the participants filled Roland-Morris Disability Questionnaire (Supplement 2) to record a patient's self-rated physical disability caused by low back pain a self-made low back pain questionnaire (Supplement 3) to determine the nature of the low back pain.[88] As previously mentioned, we have taken the medical history from all participants and none of them have been diagnosed with lumbar spine disease previously. Accordingly, we did not make a specific, neurological physical examination for low back pain before the MRI examination.

Exclusion criteria included acute heart failure, pregnancy, breastfeeding, those with a positive pregnancy test, immunosuppressive/immunomodulatory therapy in the last 30 days including systemic steroid-containing medicines, chemotherapy in the last year, major surgery in the last 2 months, transfusion, receiving other blood products in the last 2 months, pacemaker, Implantable Cardioverter-Defibrillators (ICD) other implanted device, magnetisable metal object in the body, claustrophobia, and aphasia.

Comorbidities were derived from subject reports and their medications. If both members of the twin pair were eligible, only one of them was randomly selected using an online platform (https://www.sealedenvelope.com/simple-randomiser/v1/lists).

3.2.1 Lumbar MRI

After the sleep examinations, patients underwent a lumbar spine MRI on a Siemens Magnetom Verio 3T scanner at the Borsod-Abaúj-Zemplén County Hospital (Miskolc, Hungary) or on a Philips Ingenia 1.5T at the Medical Imaging Centre of Semmelweis University (Budapest, Hungary).

We performed sagittal T1-weighted, T2-weighted, short tau inversion recovery and axial T2-weighted imaging. Two experienced radiologists accomplished the grading on T2 weighted and STIR images retrospectively, and the axial sections were obtained at selected levels to assess structural changes in individuals who had features suggesting disc prolapse.

The presence or absence of disc dehydration, disc height narrowing, disc bulging, or disc herniation were noted based on the standardised criteria.[89–91] Disc dehydration was measured on sagittal T2 weighted images. If the disc signal intensity was lower compared to that of the cerebrospinal fluid, disc dehydration was established, but we did not differentiate the varying levels of dehydration. As previously mentioned, disc narrowing was determined if the height of the disc proved to be equal or lower than the one directly cranial to it on sagittal T1 weighted images. In the case of the 5^{th} lumbar disc, narrowing was ascertained if its height was lower than that of the 4^{th} lumbar disc. Bulging or herniation was recorded on axial T2 weighted images. Bulging was noted if the intervertebral disc material extended beyond the space between the vertebral bodies and this was more than 25% of the disc's circumference; herniation was registered if less than 25% of disc circumference. These two abnormalities were counted and summarised as a total value between the 12^{th} thoracal and 1st sacral segments. Endplate degeneration was measured on T1 weighted scans, and their status was categorised into six types according to severity of damage (type 1 to type 6). Total endplate score was calculated by summing the endplate defect scores of both rostral and caudal endplates of the disc.[61]

Pfirrmann's grading (Pfirrmann score) was used to assess disc degeneration on T2 weighted images. If the discs were Grade I, II or III, we considered them to be healthy; if

discs were Grade IV or V, they were classified as degenerated.[92] The total score for the entire lumbar spine also calculated by adding each disc's Pfirrmann score.

In the process of disc and small joint degeneration, the vertebral position can change. The position of a vertebra is relative to the one below, so in anterolisthesis the vertebra slips forward, and in retrolisthesis its displacement is posterior.[63]

On T1 weighted scans the superior and inferior endplate disease were measured and classified into 4 categories: having no abnormality (0), mild (1 to 5 mm), moderate (5 to 10 mm) and severe (over 10 mm) defects. The average score was then calculated for the entire lumbar spine.

The presence of a Schmorl node was also recorded in the superior and inferior vertebra on T1 or T2 weighted scans. The presence of anterior and posterior spondylophytes was noted. On T1-weighted images vertebral fatty degeneration was evaluated and classified as none (0), mild (1, up to 25% fatty infiltration), moderate (2, 25–50% fatty infiltration) or severe (3, 50–100% fatty infiltration) and summed to create a total score for the lumbar spine. High intensity zones - a high-intensity focal signal on T2 weighted sequences in the posterior anulus fibrosus—were marked.[72]

To further clarify the association between OSA and lumbar degeneration, patients with osteoporosis and those who were on analgesic treatment were excluded. A total of 13 participants had to be excluded based on the criteria detailed above. The statistical analysis was performed on the full sample and the restricted sample (exclusion of people with osteoporosis and people taking painkillers).

3.3 Statistical analysis

3.3.1 Sleep Study

Descriptive statistics (mean ± standard deviation for continuous variables, percentage for categorical variables) were computed. AHI, RDI, and ODI were log-transformed due to non-normal distribution, and then linear and logistic regression analyses were used to identify variables independently associated with them.

Structural equation modelling was performed to estimate the variance components of the ACE model, which partitions whether variance is due to additive genetic effects (A or h2: heritability estimates), common environmental effects (C or c2: proportion of variance explained by common environments), or other residual effects (E or e2, including SE: standard error) (Figure 23). The effects of genes at multiple loci or multiple alleles at one locus were measured by A, the common family environment of both twins (diet, exposure to high levels of air pollution, familiar socialisation, shared womb, etc.) was counted in C, and other effects that apply to only individual twins including measurement error were assessed in E. If A was 0, the CE model was used.[93] Heritability analyses were conducted using the variance component models implemented in SOLAR Eclipse version 8.1.1 by the members of the Korean Twin Registry, which analyses the differences in variances according to family structure.[93] Univariate heritability was estimated to assess the heritability of each of the single traits. Heritability was estimated using the proportion that is explained by additive genetic effects over the total phenotypic variance, after adjusting for potential confounding factors such as age and sex as fixed effects. Post-hoc adjustment for BMI has also been performed. C was modelled based on family IDs.

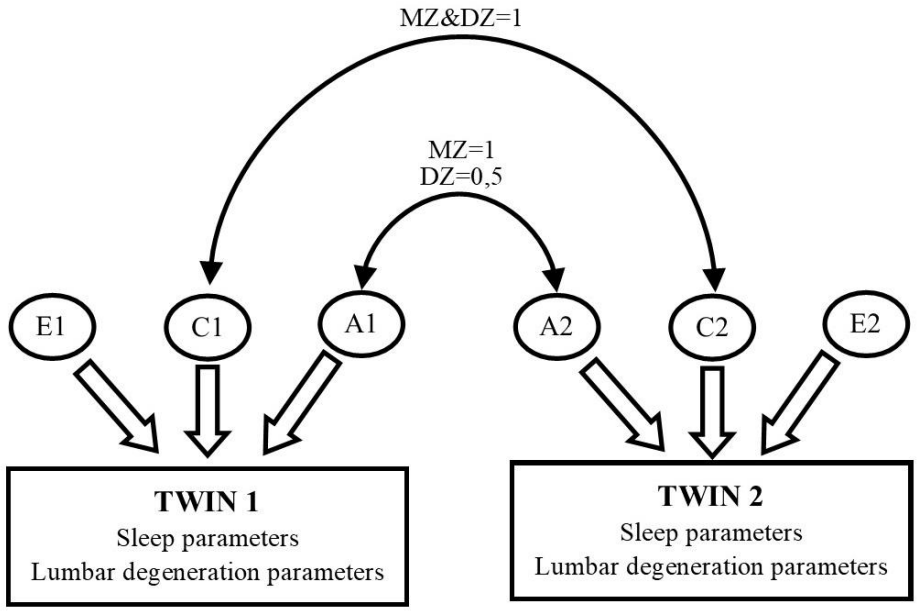

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Figure 23: Schematic diagram of the ACE model:

Monozygotic twins have the same genetic and common environmental background; conversely, dizygotic twins share only 50% of their genetic material on average, so we can use this difference to infer the genetic determinants of the factors under study (author's own figure).

3.3.2 Study on the association of OSA with lumbar spine MR abnormalities

Statistica 12 (StatSoft, Inc., Tulsa, OK, US) software was used for analysis. The normality of the data was assessed with the Kolmogorov–Smirnov test. T-test, Mann–Whitney, Fisher, and Chi-square tests were used to compare the OSA and control groups. Comparisons of back pain and MRI results were adjusted for age, gender, BMI, smoking history, cardiovascular and cerebrovascular diseases, and the type of sleep test. Sleep parameters were correlated with outcomes of pain questionnaires and MRI parameters using Spearman's correlation (continuous variables), negative binomial model (count variables), and logistic regression (dichotomous variables). The latter two analyses were adjusted for gender, age, BMI, cardiovascular disease, smoking, and the type of sleep test. The relationship between discopathy and chronic pain was assessed with Spearman's correlation, logistic regression analysis, or a negative binomial model following adjustment for age, gender, BMI, and ODI. The relationship between klotho levels in lumbar spine pathology was assessed with Spearman test, logistic regression, or a negative binomial model adjusted for gender, age, BMI, and ODI. The analyses were performed after excluding patients with osteoporosis and those taking painkillers ($n = 13$). A p value < 0.05 was considered significant. Data are presented as mean $\pm$ standard deviation or median/interquartile range/.

The primary aim of the study was to analyse the relationship between OSA and lumbar spondylosis. As the results were obtained from a limited population, no formal power analysis was performed. Post-hoc sensitivity analyses showed that we were able to detect an effect size greater than 0.67 with a power of 0.80 and an alpha error of 0.05.

4 Results

4.1 Sleep Study

4.1.1 Clinical characteristics and measures

As shown in Table 3, 68% of all twins were monozygotic and 32% were dizygotic, with females representing 70% of the study sample. The subjects had in average normal BMI (mean BMI: 24.1 kg/m^2). Diabetes was detected in 14 participants, hypertension in 60 participants, and hypercholesterinaemia in 55 participants. OSA was diagnosed in 58 subjects (41%), of whom 44 had mild (AHI 5-15/h), 12 had moderate (AHI 15-30/h), and 2 had severe (AHI> 30/h) disease. Patients with moderate-to-severe disease were offered continuous positive airway pressure therapy, however, their progress has not been evaluated as part of this study.

Table 3: Characteristics of the study population (n=142)

Characteristics	Mean(SD)/N(%)
Number of twins	
MZ twins	48 pairs (67.6 %)
DZ twins	23 pairs (32.4%)
Sex	
Male	40 (28,2 %)
Female	102 (71.8 %)
Age [years]	50.5 (15.5)
Body mass index (BMI) [kg/m^2]	24.1 (3.6)
Diabetes mellitus (DM)	16 (11.30 %)
Hypertension	60 (42.3 %)
Hypercholesterinaemia	55 (39.3%)
Epworth sleepiness scale (ESS)	6.2 (3.6)
Apnoea hypopnoea index (AHI) [events/hour]	6.4 (7.0)
Arousal index (AI) [events/hour]	48.0 (21.3)
Respiratory arousal index [events/hour]	1.33 (1.77)
Minimal oxygen saturation (Min$SatO_2$)	88.5 (4.6)
Oxygen desaturation index (ODI) [events/hour]	5.0 (6.8)
Respiratory disturbance index (RDI) [events/hour]	14.7 (8.7)
Total Sleep Time (TST) [day]	0.27 (0.03)
TST90% [%]	2.3 (6.1)
Presence of obstructive sleep apnoea (OSA) based on AHI>5/h	58 (41.4 %)

Tables 4 and 5 show the regression analysis with risk factors associated with obstructive respiratory indices (logAHI, logRDI, logODI). There was a significant relationship between BMI and RDI as well as ODI (both $p<0.01$) with a trend for a significant association between BMI and AHI ($p=0.07$). Age was directly related to all of the indices (all $p<0.01$). Only higher ODI was associated significantly with the male gender, while AHI or RDI did not correlate with gender. There was no relationship between smoking history and obstructive respiratory events (all $p>0.05$).

Table 4: Association of parameters related to sleep disturbance with metric risk factors

	logAHI		**logRDI**		**logODI**	
	R^2	**p**	**R^2**	**p**	**R^2**	**p**
age	**0.096**	**<0.0001**	**0.070**	**0.002**	**0.290**	**<0.0001**
BMI	0.086	0.074	**0.210**	**0.003**	**0.277**	**0.001**

This table shows the determination coefficient of regression, R2, and related p value

Table 5: Association of parameters related to sleep disturbance to categorical risk factors

	logAHI		logRDI		logODI	
	Wilks'-lambda	**p**	**Wilks'-lambda**	**p**	**Wilks'-lambda**	**p**
Sex	0.984	0.206	0.984	0.162	0.963	0.032
Smoking	0.988	0.216	0.979	0.107	0.997	0.539

The calculated values are Wilks'-lambda and associated p-value

4.1.2 Univariate genetic modelling

The comparison of h2 with c2 proved genetic influence in all variables except for TST, suggesting that genetic factors may be important contributors to these variables (Table 6). We fitted ACE models, in which variance of the observed total phenotypes was partitioned into additive genetic, common environmental, and other random effects. Heritability was estimated using proportion explainable by additive genetic effects over the total phenotypic variance, after adjusting for potential confounding factors such as age and sex as fixed effects. Univariate heritability was estimated to assess the heritability of each of the single traits, and bivariate heritability estimations were conducted to assess genetic and environmental correlations between two variables of interest.

For most parameters, the best fitting model was one including the A (h2) and E (e2) components, because the C (c2) does not explain any proportion of the variance, except the Epworth Sleepiness Scale where the ACE model was used. Heritability could not be detected in cases of TST, so the CE model had a better fit. (Table 6) The heritability of AHI is 73%, of ODI is 83%, and of RDI is 69%, showing that strong heritability is in the range of 69% to 83%, while the OSA itself was 73% heritable. The unshared environmental component explained the rest of the variance between 17% and 31%. Shared environmental effects for these measures were not detected. The Epworth Sleepiness Scale was mostly determined by the environment, and the variance was

influenced by 34% by the additive genetic factors. Additional adjustment for BMI besides sex and age in Model 2 did not influence the results.(Table 6)

Table 6: Heritability estimates of breathing-related sleep disorder indices in a case of Model 1 and Model 2

	h^2 (SE)	**c^2 (SE)**	**e^2 (SE)**
AHI	0.73 (0.08)***	0	0.27
AI	0.55 (0.13)***	0	0.45
ESS	0.34 (0.38)*	0.31 (0.36)	0.66
$MinSatO_2$	0.96 (0.01)***	0	0.04
ODI	0.83 (0.05)***	0	0.17
OSA	0.73 (0.15)***	0	0.27
RDI	0.69 (0.09)***	0	0.31
RespAI	0.75 (0.16)*	0	0.25
TST	0	0.25 (0.14)*	0.75
TST90	0.97 (0.008)***	0	0.03

*p<0.2, **p<0.05, ***p<0.001

Model 1 for age and sex, Model 2 for age, sex and BMI are adjusted

h^2: heritability estimates; c^2: proportion of variance explained by common environments; SE: standard error, e^2: proportion of variance explained by unique environments

Model 2 results did not change after additional adjustment for BMI besides age and sex.

4.2 Study on the association of OSA with lumbar spine MR abnormalities

4.2.1 Comparison of the OSA and control groups

Among participants who underwent a lumbar spine MR scan (56 participants), OSA was diagnosed in 27 participants, and 21 had mild (AHI <15/h) disease. Patients with OSA were older and had a higher prevalence of smoking history, hypertension, cardiovascular or cerebrovascular disease, and osteoporosis (all $p<0.05$) and tended to have higher BMI ($p=0.05$). As expected, AHI, ODI, and TST90% were higher in patients with OSA ($p<0.05$, Table 7).

Table 7: Comparison of demographics and clinical characteristics of patients with OSA and controls

	OSA (n=27)	Controls (n=29)	P
Age [years]	**60 /53-68/**	**41 /28-54/**	**<0.01**
Gender [male %]	33	28	0.64
BMI [kg/m^2]	26.6 /23.3-29.6/	24.4 /21.0-27.7/	0.05
Smoker [ever %]	26	3	0.01
Hypertension [%]	**67**	**28**	**<0.01**
Cardiovascular or cerebrovascular disease [%]	26	3	0.01
Depression [%]	**7**	**3**	**0.51**
Diabetes [%]	22	10	0.22
Dyslipidaemia [%]	48	38	0.44
Asthma [%]	11	7	0.58
COPD [%]	7	3	0.51
Osteoporosis [%]	19	0	0.01
Aspirin use	**7**	**22**	**0.03**
Opioid use	4	0	0.30
ESS	6 /4.5-10.5/	6	0.76
TST (min)	394 /378-430/	416 /362-424/	0.67
SPT (min)	435 /406-462/	425 /407-437/	0.41
Sleep %	95 /89-98/	96 /92-99/	0.32
AHI (1/h)	**9.4 /6.5-14.2/**	**2.3 /1.0-2.8/**	**<0.01**
ODI (1/h)	**8.4 /4.9-11.2/**	**0.9 /0.2-1.7/**	**<0.01**
TST90% [%]	**1.3 /0.2-2.9/**	**0.0 /0.0-0.0/**	**<0.01**

Data are presented as median /interquartile range/.

4.2.2 Lumbar spine spondylosis in patients with OSA and controls

Patients with OSA had an increased number of disc bulges ($p<0.01$, after exclusion $p<0.01$) and a higher Pfirrmann score ($p<0.01$, after exclusion $p=0.05$). We detected an increased number of anterior spondylophytes ($p=0.01$) and an increased magnitude of vertebral fatty degeneration ($p<0.01$) in patients with OSA compared to the controls. However, after excluding patients with osteoporosis and those who were taking painkillers, these differences became insignificant ($p=0.07$ for the number of anterior spondylophytes, $p=0.06$ for the magnitude of vertebral fatty degeneration). There was no difference in any other degenerative parameter (Table 8).

Table 8: Characteristics of lumbar spine spondylosis and discopathy in patients with OSA and controls

	OSA (n=27)	Controls (n=29)	P
Disc hernia (at least one, %)	28	26	0.92
Number of all disc hernias (n)	0.6±1.0	0.3±0.5	0.92
Total endplate score (n)	20.9±7.8	20.8±9.7	0.38
Disc bulging (at least one, %)	**85**	**52**	**<0.01**
Number of all disc bulgings (n)	**4.6±3.7**	**1.7±2.5**	**<0.01**
Total Pfirrmann score (n)	**16.7±4.7**	**13.2±4.1**	**<0.01**
Anterolisthesis (at least one, %)	15	3	0.13
Anterolisthesis (mm)	0.7±1.8	0.1±0.7	0.13
Retrolisthesis (at least one, %)	12	3	0.28
Retrolisthesis (mm)	0.3±1.0	0.2±1.1	0.28
Superior endplate disease (n)	0.8±1.6	0.7±2.1	0.15
Inferior endplate disease (n)	0.8±1.3	0.7±2.0	0.44
Superior Schmorl nodes (n)	0.8±1.2	0.9±1.4	0.90
Inferior Schmorl nodes (n)	0.4±0.8	0.7±1.1	0.56
Vertebral fatty degeneration (score between 0 and 18)	**7.8±4.7**	**3.8±3.7**	**<0.01**
Anterior spondylophytes (n)	**2.7±4.2**	**0.8±2.1**	**0.01**
Posterior spondylophytes (n)	1.1±2.4	0.4±0.9	0.47
Disc height narrowing (mm)	3.3±2.1	3.0±3.2	0.15
Annular high intensity zone (n)	0.6±1.2	0.3±0.6	0.40

Analysing all subjects together, there were significant correlations between the number of disc bulges and markers of OSA severity, such as AHI, ODI, and TST90% (all $p<0.05$) as well as the number of anterior spondylophytes and AHI, ODI, and TST90% (all $p<0.05$). These associations were significant (all $p<0.05$) even when the 13 subjects were excluded except for the relationship between AHI and anterior spondylophytes ($p=0.07$). When only patients with OSA were analysed, significant relationships were present only with markers of overnight hypoxaemia (Figures 24 and 25). Of note, a direct relationship was observed between the number of disc bulges and the number of anterior spondylophytes ($\rho=0.54$, $p<0.01$, after exclusion $\rho=0.51$, $p<0.01$). This relationship was present even following further adjustment for the presence of OSA.

The number of posterior spondylophytes correlated only with ODI ($\rho=0.32$, $p=0.01$, after exclusion $\rho=0.38$, $p=0.01$). The Pfirrmann score was significantly related to AHI ($\rho=0.40$, $p<0.01$, after exclusion $\rho=0.34$, $p=0.03$), ODI ($\rho=0.56$, $p<0.01$, after exclusion $\rho=0.49$, $p<0.01$) and TST90% ($\rho=0.61$, $p<0.01$, after exclusion $\rho=0.59$, $p<0.01$). There was a significant relationship between the disc height narrowing and ODI ($\rho=0.28$, $p<0.05$). However, after excluding patients with osteoporosis and those who were taking painkillers, this became insignificant ($\rho=0.21$, $p=0.18$). Disc height narrowing significantly correlated with TST90% ($\rho=0.29$, $p<0.05$, after exclusion $\rho=0.31$, $p=0.05$). None of the other sleep parameters or ESS correlated with any characteristics of lumbar spondylosis (all $p>0.05$).

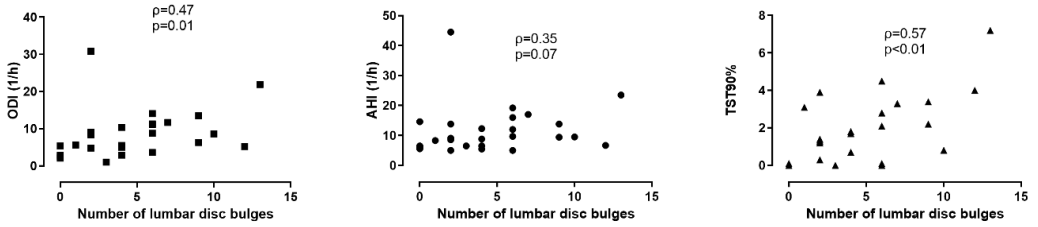

Figure 24: The relationship between the number of lumbar disc bulges and markers of OSA severity

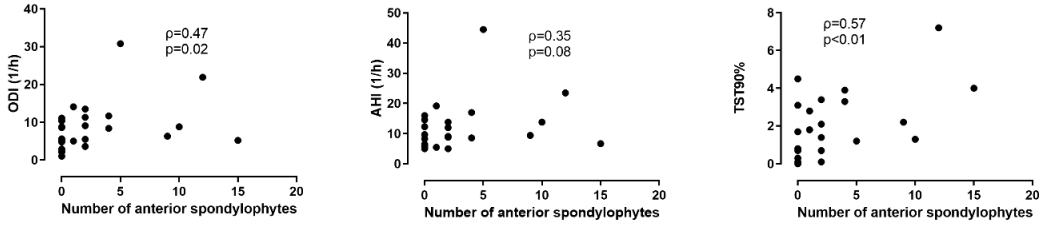

Figure 25: The relationship between the number of anterior vertebral spondylophytes and markers of OSA severity.

4.2.3 Chronic pain in patients with OSA and controls

There was no difference in the lifetime prevalence of lower back pain between the OSA (85%) and control groups (72%, p=0.78, after exclusion p=0.85). Thirty-eight per cent of the patients and 34% of controls reported lower back pain in the last 4 weeks (p=0.41, after exclusion p=0.77). Fifty-five per cent of the patients and 28% of controls reported that lower back pain limits their daily activities (p=0.56, after exclusion p=0.32). However, there was no difference in the RMDQ score between the two groups (0 /0-3.5/ vs. 0 /0-1/; OSA vs. controls, respectively, p=0.29, after exclusion p=0.25). Similarly, no significant differences were found regarding responses to other questions between the two groups (all p>0.05).

None of the sleep parameters related to the lifetime prevalence of lower back pain or lower back pain in the last 4 weeks (p>0.05, after exclusion p>0.05). There was a tendency for a direct relationship between RMDQ and ESS (ρ=0.27, p=0.07). After excluding the patients with osteoporosis and those who were taking painkillers, this

relationship became significant (ρ=0.38, p=0.03). There was no relationship between chronic pain and BMI (p>0.05, after exclusion p>0.05).

4.2.4 The relationship between lumbar spine spondylosis, discopathy, and chronic pain

Participants who had any disc bulging had a higher RMDQ score (1 /0-3/ vs. 0 /0-0/, p=0.01, Figure 26) even after excluding the 13 subjects (p=0.03). Interestingly, there was no relationship between the number of disc bulges and RMDQ (p=0.20) or the other pain outcomes (p>0.05).

There was a significant relationship between the Pfirrmann and the RMDQ score (ρ=0.32, p=0.02). After excluding the patients mentioned above, this relationship became insignificant (ρ=0.09, p=0.56). There was also a significant relationship between the RMDQ score and the incidence of superior endplate disease and inferior endplate disease as well as the number of anterior spondylophytes (all p<0.05). After excluding patients, only the relationship between RMDQ and the number of anterior spondylophytes remained significant (ρ=0.46, p<0.01). None of the other characteristics of spondylosis or discopathy were related to measures of chronic pain.

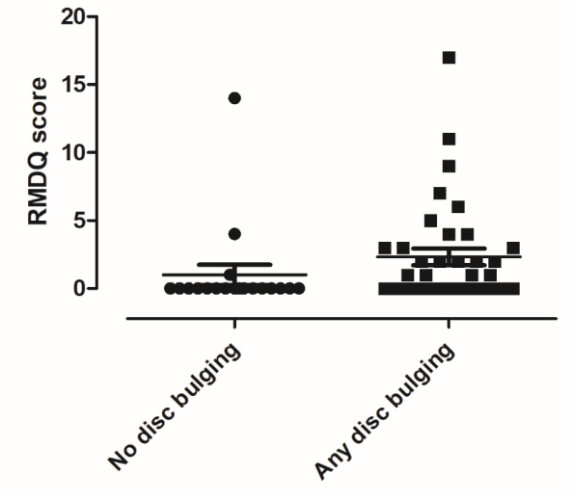

Figure 26: The Roland-Morris Disability Questionnaire score in subjects with and without lumbar disc bulges

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4.2.5 The role of klotho in lumbar spine spondylosis and discopathy associated with OSA

Plasma klotho levels were inversely related to the number of disc bulges (ρ=-0.47, $p<0.01$, after exclusion ρ=-0.49, $p<0.01$, Figure 27), and there was a tendency for an inverse correlation with total endplate score (ρ=-0.26, p=0.07) as well as annular high intensity zones (ρ=-0.27, p=0.06). These remained insignificant after excluding the patients mentioned above (all $p>0.05$). However, there was no further relationship with any other characteristics of spondylosis or discopathy (all $p>0.05$).

There was no relationship between plasma klotho and BMI (p=0.33) or age (p=0.09), while it significantly related to AHI (ρ=-0.33, p=0.03), ODI (ρ=-0.33, p=0.02) and TST90% (ρ=-0.44, $p<0.01$). Although klotho levels did not differ between the OSA and control groups (p=0.15), OSA patients with at least one anterior spondylophyte had significantly lower klotho levels compared to the controls with at least one anterior spondylophyte ($p<0.01$). However, there was no difference in klotho levels between the two groups in those patients who had at least one disc bulging (p=0.93).

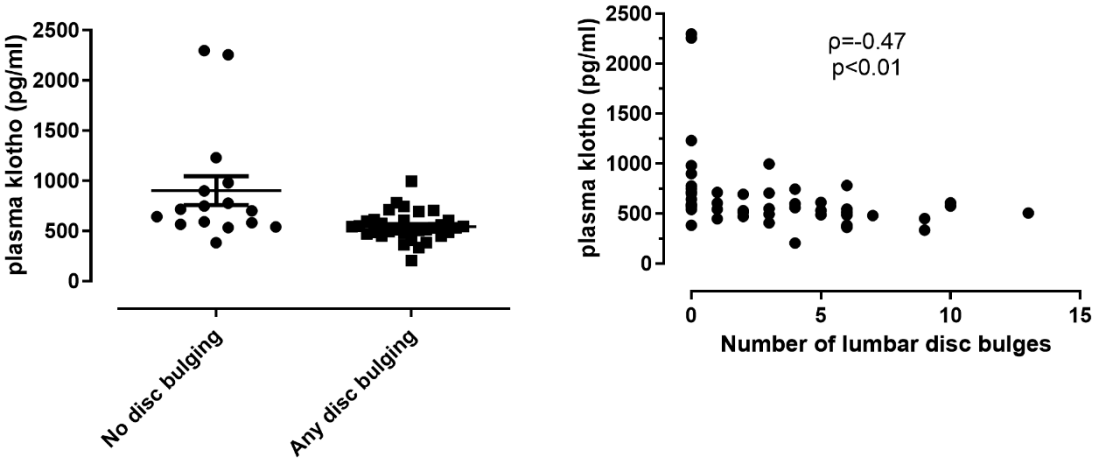

Figure 27: The relationship between plasma klotho levels and lumbar disc bulges.

5 Discussion

This is the first study investigating the heritability of OSA using objective sleep assessment. We reported that the sleep parameters and OSA show high heritability, and unshared environmental factors explain the rest of the variance. Adjustment for BMI, age, or gender implied that the influence of heritability is independent of any potential influence of these variables.

Secondly, according to our knowledge, this is the first study examining the relationship between obstructive sleep apnoea and lumbar spine abnormalities. We found that OSA was significantly associated with the presence of certain abnormalities, most strongly with disc bulges and anterior spondylophytes.

5.1 The inheritance of OSA

To begin, by comparing the twin correlations in the OSA twin study we could estimate the variance explained by additive genetic, shared environmental (e.g., shared womb) and non-shared environmental effects (e.g., lifestyle factors, measurement error). Our results can be partially explained by the inheritance of the smallest surface diameter of the oropharynx, one of the important determinants of OSA, which has been demonstrated in a family study (30-40%), although the rate of heritability was less than that we have reported.[21] The importance of anatomical variation in heritability is confirmed by volumetric MRI in a family study, which detects a significant level of heritability of the volume of the lateral pharyngeal wall, tongue, and total soft tissue.[94] The heritability of these factors may increase the heredity level in addition to the aforementioned anatomical situation. In another family study, the heredity of the AHI was 34-37%, but our twin study found a much higher inheritance.[20] The reason for this is also to be found in the different test methods because family studies are suitable for determining the similarity or difference between generations and do not take into account external factors such as family environment and culture, which in turn are taken into account in twin studies by separating genetic and common environmental factors.[86] The only previous twin study of OSA proved it to have an inheritance of around 50%. However, the OSA diagnosis was based on a questionnaire survey, which might increase the measurement error (E variance).[24] Therefore, our present study is the first to investigate the inheritance of OSA with objective overnight PSG, and the first to show high inheritance.

5.2 The consequences of high heritability and their clinical significance

High inheritance draws attention to the likelihood that the disease might occur in close relatives and descendants of patients with sleep disorders. For this reason, prevention of predisposing, controllable factors such as obesity (e.g., thick neck circumference), smoking, high blood pressure, and diabetes is essential. Non-influenced factors include male gender, ethnicity, and age. Treatment is further complicated by hereditary predisposition to body composition, the incidence of smoking and blood pressure, in 79% and 51%, according to the previous twin studies.[95–97] This means that the prevention of these predisposing factors is also important since in genetically predisposed individuals it can be associated with the formation of OSA. Most of these factors are influenced, apart from the genetic predisposition, by epigenetic factors playing an important role in transcriptional and post-transcriptional regulation, including deoxyribonucleic acid (DNA) methylation, histone acetylation, miRNA and transcriptome profiling, non-coding RNA regulation, and RNA editing.[98,99] Due to the high public health importance of OSA, close relatives of patients with OSA should be screened to prevent OSA-related emergence of comorbidities and mortality.[100] Recent publications have demonstrated the utility of OSA screening in Type 2 diabetes and obesity[101,102]. Our study draws attention to the fact that screening programs may include family history.[87]

5.2.1 The importance of obesity in OSA

It is evident that obesity, which has a high heritability itself,[95,97] predisposes to OSA, and the prevalence of OSA is increasing worldwide because of the ongoing obesity epidemic [103]. The parapharyngeal fat pad which is connected with obesity may play an important role in the development of OSA in overweight participants. Interestingly, a weight reduction program reduces the size of parapharyngeal fat pads and makes improvements in OSA.[104] Obesity-associated OSA has been independently associated with the surrogate markers of cardiovascular risk, including sympathetic activation, systemic inflammation, and endothelial dysfunction,[103] however, it was unclear whether this association is genetically linked. Our findings show evidence that BMI has associated with OSA through a non-genetic link which might have a clinical implication that physical activity or any other measure of weight reduction can be more effective in OSA management. A recent study has shown that a combined occurrence of obesity and OSA may interact to reduce exercise capacity, highlighting the importance of obesity

control programs among women.[105] This finding is supported by a recent study demonstrating that OSA patients are less physically active than individuals without OSA.[106] The high heritability of sleep parameters highlights the role of earlier identification of OSA in genetically predisposed individuals, since treatment strategies to improve sleep may contribute to overall health outcomes for patients with obesity.[107] The non-genetic link can be the reason why recent studies showed that effective treatment of OSA with CPAP significantly reduces visceral fat.[103,108]

5.2.2 Relationship between OSA and the cardiometabolic system

The cardiometabolic consequences of obesity are higher in patients with OSA, so the diagnosis and treatment of OSA are very important, especially in patients with frequent snoring and difficult-to-control hypertension, nocturnal blood pressure abnormalities, atrial fibrillation, or left ventricular hypertrophy.[103] OSA is a poorly diagnosed disease. In our study, no participant had a known breathing disorder, but after the examination 58 participants were diagnosed with OSA (44 had mild (AHI 5-15/h), 12 had moderate (AHI 15-30/h) and 2 had severe (AHI> 30/h) disease). CPAP therapy—the first-choice treatment—significantly improves the cardiometabolic status. After CPAP therapy, the blood flow in micro-and macrocirculation is improved, arterial elasticity is increased, and arterial stiffness is decreased. Furthermore, endothelial disorder biomarkers like calcium-activated potassium channels (Big Potassium, BK channels) are also lower after the therapy.[109] Sharma et al. demonstrated that CPAP therapy alone can reduce visceral adiposity and BMI, which can be explained as a consequence of partial metabolic improvements.[110] In contrast, other studies could not detect any changes in these metabolic parameters. [103]

5.2.3 Daytime sleepiness in OSA

The average daytime sleepiness reported by the study participants was not clinically significant, and even in patients with moderate to severe OSA sleepiness was not sufficiently alarming to make OSA diagnosis available prior to our study. This is in line with a very recent German population-based study that the proportion of sleepy (ESS≥10) patients within the OSA group are relatively low (15%).[103][44] In line with this, unlike OSA, sleepiness was determined by environmental factors. We did not intend to evaluate these factors, but they may include poor sleep hygiene, work shifts, diet, and medications.

5.3 The link between OSA and lumbar degeneration

As previously mentioned, chronic pain especially in the lumbar region is very common in patients with OSA[38], but interestingly previous studies focusing on the cervical spinal region reported an increased number of spondylophytes in OSA[44] and suggested that these may contribute to the development of sleep apnoea.[44] Spondylophytes are bony projections that form along joint margins. Usually, they are associated with the degeneration of the lumbar disc which starts losing water content and leads to a decrease in the intervertebral disc height. To compensate for the loss of flexibility of the anulus fibrosus, the apophyseal joints start to grow a new bone.(43,111) In line with this, we found a significant direct relationship between the number of spondylophytes and disc bulges. However, the design of this study cannot answer the causality of the relationship as both abnormalities may have been independently driven by OSA. Our study extends the previous observations on the cervical spine region to the lumbar region, as the number of disc bulges and spondylophytes were higher in OSA and related to disease severity.

5.3.1 Possible mechanisms explaining the relationship between the two diseases

The relationship between OSA and disc degeneration can be explained by multiple mechanisms. Several genes of collagen biosynthesis and inflammation were found to be associated with OSA [112] and disc degeneration[113], also suggesting a common genetic origin. Age, male gender and obesity, and risk factors for OSA[114] are associated with an increased likelihood for the development of disc degeneration.[113],[115] Of note, the relationship between OSA and discopathy in the present study was present after adjustment for these factors. OSA is also associated with abnormal bone formation, likely due to the accelerated remodelling induced by intermittent hypoxia[46] and vitamin D deficiency in sleep apnoea.[48]

As previously mentioned, the apoptosis and senescence of disc cells (anulus fibrosus and nucleus pulposus) and the structure of the matrix are both crucial in the development of degeneration. Chronic intermittent hypoxia in OSA leads to oxidative stress and inflammation[114], and as a result, ROS production has increased. ROS production has proapoptotic effects in NP and AF cells in rabbits[116] and promotes premature senescence of AP and AF cells.[113] Furthermore, the ROS cause oxidative modification of the collagen structure which leads to its crosslink, aggregation and unfolding.[117]

This modification of the collagens significantly impairs its anatomical integrity and functional property.[118]

Furthermore, reactive oxygen species can induce the expression of extracellular matrix proteases and proinflammatory markers in disc cells.[119] Supporting this, a direct relationship was seen between markers of overnight hypoxaemia and markers of disc degeneration in the current study. In addition, systemic inflammation generated in OSA may also contribute to inflammation in lumbar discs.[120]

A potential molecule that is involved in the pathomechanism of both diseases is the klotho protein.[77,83] This protein is synthesized predominantly in the kidneys, but other organs, such as parathyroid gland, ovary, testis and placenta also express the klotho gene.[121] Klotho is involved in phosphate homeostasis, regulates aldosterone synthesis, and has anti-aging, anti-oxidative stress and anti-inflammatory role.[78] We have reported low levels of klotho in OSA which were associated with markers of overnight hypoxaemia.[77] Hypoxaemia[122] and oxidative stress[83] lead to decreased klotho expression in nucleus pulposus cells. Klotho attenuates inflammation at the same site[83] and it is hypothesized that lower klotho levels contribute to disc degeneration.[83] Furthermore, klotho gene polymorphisms were associated with lumbar spondylosis.[82]

5.3.2 Chronic pain and OSA

As in previous studies, [123] we did not find a significant difference in reported back pain between patients with OSA and controls. In contrast, back pain was associated with increased daytime sleepiness. Chronic pain can disturb the normal sleep architecture[41] with subsequent daytime effects. Although excessive daytime sleepiness is a common symptom of OSA[123], our study highlights that other factors besides OSA can lead to this symptom, and these should be explored in clinical practice. Most particularly, clinicians should not accept chronic pain as a natural component of fibromyalgia syndrome, but may instead investigate anatomic causes for pain and sleepiness. In contrast to OSA, chronic pain was associated with lumbar spondylosis and discopathy. This may highlight an indirect mechanism between spondylosis and OSA development, as chronic pain may limit mobility, eventually leading to weight gain. Obesity is a leading cause of OSA, and limited exercise could subsequently cause OSA development. A previous meta-analysis concluded that obesity is significantly associated with low back

pain[123]; our study however did not find any relationship between BMI and pain outcomes. Notably, due to the subjective nature of the outcome and the limited number of subjects, the results need to be interpreted carefully. [88]

5.4 Limitations

Limitations of our study should be considered. First, the relatively small sample size and especially a low number of DZ pairs prohibited a more precise evaluation of each sleep-associated variable (e.g., TST) and lumbar parameters. Increasing the number of patients could allow more complex relationships between lumbar degeneration and sleep parameters to be detected.

Second, the study population consisted of patients with relatively mild disease severity. Further twin studies involving patients with morbid obesity or more severe OSA are warranted to see if this high genetic association is present in these patients. Finally, the heritability model based on twins has been criticized because the gene-environment interplay is very difficult to assess.[43] However, large-population twin studies can take into account gene–environment and gene–gene interactions in order to study complex phenotypes. Furthermore, patients with more severe diseases could reveal more precisely the relationship between OSA and lumbar spine degeneration. More importantly, the lack of relationship between OSA and chronic pain could be due to the milder disease severity. Interestingly, the relationship between OSA and disc bulges as well as spondylophytes was evident even at this severity.

Third, two different MRI machines were used. and some patients had cardiorespiratory polygraphy instead of polysomnography. Polygraphy may underestimate the severity of OSA, as hypopnoeas associated with arousals but without desaturations are not counted, and the total number of respiratory events is divided by the total time recorded rather than the total sleep time. Acknowledging these biases, the results were adjusted for the type of sleep test.

Fourth, the groups were not balanced in terms of age, gender, BMI, and comorbidities. Although the analyses were adjusted for these factors, further studies in larger populations are warranted to minimize this bias.

Fifth, physical activity may influence the results, however, this has not been recorded in this study.

Sixth, none of the patients were treated with continuous positive airway therapy. Follow-up trials are warranted to see whether CPAP could be beneficial in preventing or slowing down spondylosis in patients with OSA. Interventional trials may also reveal independent associations between OSA and spondylosis not biased by confounders. Despite these limitations, we believe that our pioneering study may serve as a potential basis for designing large-scale studies.

Seventh, there was no physical examination before the lumbar spine MRI and the compression, dislocation, and involvement of spine nerves were not examined in the MRI images. None of our participants has been diagnosed with low back pain or lumbar degeneration confirmed by MRI, therefore, the presence of lumbar spine root lesions was not assumed. In a further study, these lesions should be examined to assess more accurately the lumbar degeneration and chronic low back pain.

Eight, in our study the anterior spondylophytes were more common than the posterior ones and anterior spondylophytes showed correlation with the sleep parameters. A previous study also showed that the spondylophytes in the anterior surfaces are more common than the posterior. This difference in the location of the spondylophytes can be explained by biomechanical factors. For example, spondylophytes are located more frequently at the concavity of scoliotic curves.

6 Conclusions

According to our knowledge, this was the first study examining Obstructive Sleep Apnoea with objective methods in twins in addition to the link between OSA and lumbar degeneration in twins.

The present study showed objectively for the first time the strong genetic effects on obstructive sleep apnoea variables in adult healthy twins, independently of age, gender, and body mass index. Anatomical abnormalities and variabilities play a key role in this with high probability, so our research group has conducted further studies to quantify the heritability of cervical region anatomy. The relatively low unshared environmental influence still highlights the role of the prevention of known environmental risk factors, particularly through epigenetic effects. These observations provide important insights into the pathogenesis and potential treatment of OSA and stimulate further epigenetic studies to understand interconnections between the pathophysiology of sleep and metabolic diseases, including obesity.

Our research has shown that the number of disc bulges and spondylophytes was higher in OSA and was related to disease severity in patients with OSA. Our study cannot answer the casualty of this connection, but in the background, several genes of collagen biosynthesis and inflammation were found to be associated with OSA [112] and disc degeneration.[113] In addition to common risk factors (age, male gender, obesity), vitamin D deficiency and chronic intermitting hypoxia-caused by reactive oxygen species and systematic inflammation may be involved in the common pathogenetic pathways.

The connection between OSA and spondylosis calls attention to chronic pain in the lumbar region in patients with OSA. We cannot show a significant difference in the reported back pain between patients with OSA and controls, but the chronic pain disturbs the normal sleep architecture with subsequent daytime effects (daytime sleepiness) which are the common symptoms in OSA. Because of the chronic pain associated with spondylosis and discopathy, clinicians should not accept chronic pain as a natural component of fibromyalgia syndrome but may investigate anatomic causes for pain and sleepiness.

Kloto protein is a potential molecule in both pathomechanisms, and we found that the klotho protein level was associated with the number of disc bulges and markers of overnight hypoxaemia.[77] We hypothesise that lower klotho levels contribute to disc degeneration[83] because hypoxaemia[122] and oxidative stress lead to decreased klotho expression in nucleus pulposus cells, and at the same site klotho attenuates inflammation.[83] This also supports that klotho gene polymorphisms were associated with lumbar spondylosis.[82]

7 Summary

OSA is one of the most common breathing-related sleep disorders with severe symptoms that negatively affect patients‘ quality of life. According to our knowledge, the heritability of OSA has not previously been investigated using an objective method. Our study showed that OSA is highly heritable, which may be due to heritable anatomical factors and obesity. In contrast, daytime sleepiness is more influenced by environmental factors. OSA is difficult to diagnose, and its heritability calls for the screening of close relatives of patients and the reduction of controllable factors (e.g., obesity, smoking, hypertension and diabetes). Appropriate management of OSA can reduce obesity and treatment improves cardiometabolic status in obese OSA patients, so early detection and treatment is a key factor.

Many patients with OSA suffer from chronic pain, yet lumbar degeneration, the most common cause of chronic low back pain, has not been investigated. Our study has shown that the hypoxic parameters of OSA and some features of lumbar degeneration are related. The possible mechanisms: genes responsible for collagen synthesis and inflammatory processes are both associated with OSA and lumbar degeneration, common aetiological factors (age, male sex and obesity), reactive oxygen species and chronic inflammation induced by chronic intermittent hypoxic episodes and impaired vitamin D metabolism. At the molecular level, klotho protein may also explain this association, in our study, its levels were low in OSA and associated with hypoxic parameters. We hypothesize that the lower levels of klotho make its anti-inflammatory effect less pronounced, which promotes lumbar degeneration. No significant difference was found between the OSA and healthy groups in terms of LBP, but LBP and daytime sleepiness were associated. Therefore, it is important to identify the cause of LPB in patients with OSA and to consider the possibility of OSA in patients with poor sleep in LBP..

8 Összefoglalás

Az OSA az egyik leggyakoribb légzéssel összefüggő alvási rendellenesség, melynek komoly, életminőséget negatív irányba befolyásoló tünetei vannak. Szakirodalmi adatok alapján korábban az OSA örökletességét eszközös módszerrel nem vizsgálták. Vizsgálatunk során kimutattuk, hogy az OSA nagyfokú örökletességet mutat, melynek hátterében az örökölhető anatómiai faktorok és az elhízás állhat. Ezzel szemben, a nappali álmosság inkább környezeti tényezők által befolyásolt. Az OSA nehezen diagnosztizálható eltérés, így örökletessége felhívja a figyelmet a betegségben szenvedő egyének közeli rokonainak szűrésére, illetve a befolyásolható faktorok (pl. elhízás, dohányzás, magas vérnyomás és cukorbetegség) csökkentésére. Az OSA megfelelő kezelése képes mérsékelni az elhízást és az elhízott OSA-s betegek esetén a kezelés javítja a kardiometabolikus állapotot, így korai felismerése és kezelése kulcsfontosságú.

Számos OSA-s beteg kűzd krónikus fájdalommal, ennek ellenére a lumbális degenerációt, mint a krónikus derékfájdalom leggyakoribb okát nem vizsgálták. Kutatásunk során láthattuk, hogy az OSA hypoxiás paraméterei és a lumbális degeneráció egyes jellemzői kapcsolatot mutatnak. Egyrészről, ennek hátterében a mindkét betegség kialakulásában szerepet játszó, kollagénszintézisért és gyulladásos folyamatokért felelős gének, valamint a közös etiológia faktorok (életkor, férfi nem és elhízás), az OSA-ban tapasztalható krónikus intermittáló hypoxiás epizódok által indukált reaktív oxigén gyök felszabadulás és krónikus gyulladás, illetve a D vitamin homeosztázis zavara is állhat. Molekuláris szinten a klotho fehérje is magyarázhatja ezt a kapcsolatot, vizsgálatunkban ennek értéke OSA-ban alacsony volt és asszociált az OSA hypoxiás paramétereivel. Feltételezésünk szerint, az alacsonyabb klotho szint miatt annak gyulladásgátló hatása kevésbé érvényesül, így hozzájárul a lumbális degenerációhoz. A gerincfájdalom tekintetében nem találtunk szignifikáns különbséget az OSA-s és az egészséges csoport között, azonban a gerincfájdalom és a nappali álmosság összefüggést mutatott. Fontos tehát, hogy az OSA-ban szenvedő betegek derékfájdalmának okát felkutassuk, illetve a rosszul alvó derékfájdalomban szenvedő betegeknél gondoljunk az OSA lehetőségére.

9 References

1. Colten HR, Altevogt BM. Sleep disorders and sleep deprivation: An unmet public health problem. Colten HR, Altevogt BM, editors. Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem. Washington (DC); 2006. 1–404 p.

2. van de Straat V, Bracke P. How well does Europe sleep? A cross-national study of sleep problems in European older adults. Int J Public Health. 2015;60(6):643–650.

3. Sateia MJ. International classification of sleep disorders-third edition: highlights and modifications. Chest. 2014 Nov;146(5):1387–1394.

4. Gharibeh T, Mehra R. Obstructive sleep apnea syndrome: natural history, diagnosis, and emerging treatment options. Nat Sci Sleep. 2010;2:233–255.

5. Punjabi NM. The epidemiology of adult obstructive sleep apnea. Proc Am Thorac Soc. 2008 Feb;5(2):136–143.

6. Eckert DJ, Malhotra A. Pathophysiology of adult obstructive sleep apnea. Proc Am Thorac Soc. 2008;5(2):144–153.

7. Patil SP, Schneider H, Marx JJ, Gladmon E, Schwartz AR, Smith PL, Patil SP. Neuromechanical control of upper airway patency during sleep Downloaded from. J Appl Physiol. 2007;102:547–556.

8. Moser RJ 3rd, Rajagopal KR. Obstructive sleep apnea in adults with tonsillar hypertrophy. Arch Intern Med. 1987 Jul;147(7):1265–1267.

9. Lyberg T, Krogstad O, Djupesland G. Cephalometric analysis in patients with obstructive sleep apnoea syndrome. I. Skeletal morphology. J Laryngol Otol. 1989 Mar;103(3):287–292.

10. Watanabe T, Isono S, Tanaka A, Tanzawa H, Nishino T. Contribution of body habitus and craniofacial characteristics to segmental closing pressures of the passive pharynx in patients with sleep-disordered breathing. Am J Respir Crit Care Med. 2002 Jan;165(2):260–265.

11. Haponik EF, Smith PL, Bohlman ME, Allen RP, Goldman SM, Bleecker ER. Computerized Tomography in Obstructive Sleep Apnea. J Comput Assist Tomogr.

1983;7(6):1133.

12. Okubo M, Suzuki M, Horiuchi A, Okabe S, Ikeda K, Higano S, Mitani H, Hida W, Kobayashi T, Sugawara J. Morphologic analyses of mandible and upper airway soft tissue by MRI of patients with obstructive sleep apnea hypopnea syndrome. Sleep. 2006;29(7):909–915.

13. Peppard PE, Hagen EW. The Last 25 Years of Obstructive Sleep Apnea Epidemiology-and the Next 25? Am J Respir Crit Care Med. 2017 Oct;

14. Eckert DJ, Lo YL, Saboisky JP, Jordan AS, White DP, Malhotra A. Sensorimotor function of the upper-airway muscles and respiratory sensory processing in untreated obstructive sleep apnea. J Appl Physiol. 2011 Dec;111(6):1644–1653.

15. Mezzanotte WS, Tangel DJ, White DP. Waking genioglossus (gg) emg in sleep-apnea patients versus normal controls (a neuromuscular compensatory mechanism). Clin Res. 1991;39:1571–1579.

16. Fogel RB, Trinder J, White DP, Malhotra A, Raneri J, Schory K, Kleverlaan D, Pierce RJ. The effect of sleep onset on upper airway muscle activity in patients with sleep apnoea versus controls. J Physiol. 2005;564(2):549–562.

17. Naughton MT. Loop gain in apnea: gaining control or controlling the gain? Vol. 181, American journal of respiratory and critical care medicine. United States; 2010. p. 103–105.

18. Patil SP, Schneider H, Schwartz AR, Smith PL. Adult Obstructive Sleep Apnea: Pathophysiology and Diagnosis. Chest. 2010;132(1):1–21.

19. Rukhadze I, Kubin L. Mesopontine cholinergic projections to the hypoglossal motor nucleus. Neurosci Lett. 2007 Feb;413(2):121–125.

20. Patel SR, Larkin EK, Redline S. Shared genetic basis for obstructive sleep apnea and adiposity measures. Int J Obes (Lond). 2008 May;32(5):795–800.

21. Patel SR, Frame JM, Larkin EK, Redline S. Heritability of upper airway dimensions derived using acoustic pharyngometry. Eur Respir J. 2008 Nov;32(5):1304–1308.

22. Chi L, Comyn FL, Keenan BT, Cater J, Maislin G, Pack AI, Schwab RJ. Heritability of Craniofacial Structures in Normal Subjects and Patients with Sleep Apnea. Sleep. 2014;37(10):1689–1698.

23. Ferini-Strambi L, Calori G, Oldani A, Della Marca G, Zucconi M, Castronovo V, Gallus G, Smirne S. Snoring in twins. Respir Med. 1995 May;89(5):337–340.

24. Desai A V, Cherkas LF, Spector TD, Williams AJ. Genetic influences in self-reported symptoms of obstructive sleep apnoea and restless legs: a twin study. Twin Res Off J Int Soc Twin Stud. 2004 Dec;7(6):589–595.

25. Abad VC, Guilleminault C. Diagnosis and treatment of sleep disorders: a brief review for clinicians. Dialogues Clin Neurosci. 2003 Dec;5(4):371–388.

26. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep. 1991 Dec;14(6):540–545.

27. Nurgul Y. A simple and validated test for detecting patients with OSA: STOP-BANG questionnaire. Ann Card Anaesth. 2021;24(3):313–314.

28. Laratta CR, Ayas NT, Povitz M, Pendharkar SR. Diagnosis and treatment of obstructive sleep apnea in adults. Cmaj. 2017;189(48):E1481–E1488.

29. Cho JH, Kim HJ. Validation of ApneaLink™ Plus for the diagnosis of sleep apnea. Sleep Breath. 2017;21(3):799–807.

30. Bartels W, Buck D, Glos M, Fietze I, Penzel T. Definition and Importance of Autonomic Arousal in Patients with Sleep Disordered Breathing. Sleep Med Clin. 2016 Dec;11(4):435–444.

31. Gami AS, Hodge DO, Herges RM, Olson EJ, Nykodym J, Kara T, Somers VK. Obstructive sleep apnea, obesity, and the risk of incident atrial fibrillation. J Am Coll Cardiol. 2007 Feb;49(5):565–571.

32. Lavie P, Lavie L, Herer P. All-cause mortality in males with sleep apnoea syndrome: Declining mortality rates with age. Eur Respir J. 2005;25(3):514–520.

33. Somers VK, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, Pickering TG, Russell R, Woo M, Young T. Sleep

Apnea and Cardiovascular Disease: An American Heart Association/American College of Cardiology Foundation scientific statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on . Circulation. 2008;118(10):1080–1111.

34. Ip MSM, Lam B, Ng MMT, Lam WK, Tsang KWT, Lam KSL. Obstructive sleep apnea is independently associated with insulin resistance. Am J Respir Crit Care Med. 2002 Mar;165(5):670–676.

35. Sahlin C, Sandberg O, Gustafson Y, Bucht G, Carlberg B, Stenlund H, Franklin KA. Obstructive sleep apnea is a risk factor for death in patients with stroke: a 10-year follow-up. Arch Intern Med. 2008 Feb;168(3):297–301.

36. D'Ambrosio C, Bowman T, Mohsenin V. Quality of life in patients with obstructive sleep apnea: effect of nasal continuous positive airway pressure--a prospective study. Chest. 1999 Jan;115(1):123–129.

37. Li Y, Vgontzas A, Kritikou I, Fernandez-Mendoza J, Basta M, Pejovic S, Gaines J, Bixler EO. Psychomotor Vigilance Test and Its Association With Daytime Sleepiness and Inflammation in Sleep Apnea: Clinical Implications. J Clin sleep Med JCSM Off Publ Am Acad Sleep Med. 2017 Sep;13(9):1049–1056.

38. Roizenblatt S, Neto NSR, Tufik S. Sleep disorders and fibromyalgia. Curr Pain Headache Rep. 2011 Oct;15(5):347–357.

39. Wolfe F, Häuser W. Fibromyalgia diagnosis and diagnostic criteria. Ann Med. 2011 Nov;43(7):495–502.

40. Herrero Babiloni A, De Koninck BP, Beetz G, De Beaumont L, Martel MO, Lavigne GJ. Sleep and pain: recent insights, mechanisms, and future directions in the investigation of this relationship. J Neural Transm. 2020 Apr;127(4):647–660.

41. Mathias JL, Cant ML, Burke ALJ. Sleep disturbances and sleep disorders in adults living with chronic pain: a meta-analysis. Sleep Med. 2018 Dec;52:198–210.

42. Pampati S, Manchikanti L. What Is the Prevalence of Symptomatic Obstructive Sleep Apnea Syndrome in Chronic Spinal Pain Patients? An Assessment of the Correlation of OSAS with Chronic Opioid Therapy, Obesity, and Smoking. Pain

Physician. 2016 May;19(4):E569-79.

43. Khan A, Than KD, Chen KS, Wang AC, La Marca F, Park P. Sleep apnea and cervical spine pathology. Eur spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc. 2014 Mar;23(3):641–647.

44. Yang TH, Xirasagar S, Cheng YF, Wu CS, Kao YW, Shia BC, Lin HC. Association of cervical spondylosis with obstructive sleep apnea. Sleep Med. 2020 Jul;71:54–58.

45. Manchikanti L, Boswell M V, Singh V, Pampati V, Damron KS, Beyer CD. Prevalence of facet joint pain in chronic spinal pain of cervical, thoracic, and lumbar regions. BMC Musculoskelet Disord. 2004 May;5:15.

46. Sforza E, Thomas T, Barthélémy JC, Collet P, Roche F. Obstructive sleep apnea is associated with preserved bone mineral density in healthy elderly subjects. Sleep. 2013 Oct;36(10):1509–1515.

47. Arnett TR, Gibbons DC, Utting JC, Orriss IR, Hoebertz A, Rosendaal M, Meghji S. Hypoxia is a major stimulator of osteoclast formation and bone resorption. J Cell Physiol. 2003 Jul;196(1):2–8.

48. Liguori C, Romigi A, Izzi F, Mercuri NB, Cordella A, Tarquini E, Giambrone MP, Marciani MG, Placidi F. Continuous Positive Airway Pressure Treatment Increases Serum Vitamin D Levels in Male Patients with Obstructive Sleep Apnea. J Clin sleep Med JCSM Off Publ Am Acad Sleep Med. 2015 Jun;11(6):603–607.

49. Haro H, Shinomiya K, Komori H, Okawa A, Saito I, Miyasaka N, Furuya K. Upregulated expression of chemokines in herniated nucleus pulposus resorption. Spine (Phila Pa 1976). 1996 Jul;21(14):1647–1652.

50. Andersson GB. Epidemiological features of chronic low-back pain. Lancet. 1999;354(9178):581–585.

51. Kerkhof GA. Epidemiology of sleep and sleep disorders in The Netherlands. Sleep Med. 2017 Feb;30:229–239.

52. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, Charlson F, Davis A, Degenhardt L, Dicker D, Duan L, Erskine H, Feigin VL, Ferrari AJ, Fitzmaurice C, Fleming T, Graetz N, Guinovart C, Haagsma J, Hansen GM, Hanson SW, Heuton KR, Higashi H, Kassebaum N, Kyu H, Laurie E, Liang X, Lofgren K, Lozano R, MacIntyre MF, Moradi-Lakeh M, Naghavi M, Nguyen G, Odell S, Ortblad K, Roberts DA, Roth GA, Sandar L, Serina PT, Stanaway JD, Steiner C, Thomas B, Vollset SE, Whiteford H, Wolock TM, Ye P, Zhou M, Ãvila MA, Aasvang GM, Abbafati C, Ozgoren AA, Abd-Allah F, Aziz MIA, Abera SF, Aboyans V, Abraham JP, Abraham B, Abubakar I, Abu-Raddad LJ, Abu-Rmeileh NME, Aburto TC, Achoki T, Ackerman IN, Adelekan A, Ademi Z, Adou AK, Adsuar JC, Arnlov J, Agardh EE, Al Khabouri MJ, Alam SS, Alasfoor D, Albittar MI, Alegretti MA, Aleman A V., Alemu ZA, Alfonso-Cristancho R, Alhabib S, Ali R, Alla F, Allebeck P, Allen PJ, AlMazroa MA, Alsharif U, Alvarez E, Alvis-Guzman N, Ameli O, Amini H, Ammar W, Anderson BO, Anderson HR, Antonio CAT, Anwari P, Apfel H, Arsenijevic VSA, Artaman A, Asghar RJ, Assadi R, Atkins LS, Atkinson C, Badawi A, Bahit MC, Bakfalouni T, Balakrishnan K, Balalla S, Banerjee A, Barker-Collo SL, Barquera S, Barregard L, Barrero LH, Basu S, Basu A, Baxter A, Beardsley J, Bedi N, Beghi E, Bekele T, Bell ML, Benjet C, Bennett DA, Bensenor IM, Benzian H, Bernabe E, Beyene TJ, Bhala N, Bhalla A, Bhutta Z, Bienhoff K, Bikbov B, Abdulhak A Bin, Blore JD, Blyth FM, Bohensky MA, Basara BB, Borges G, Bornstein NM, Bose D, Boufous S, Bourne RR, Boyers LN, Brainin M, Brauer M, Brayne CEG, Brazinova A, Breitborde NJK, Brenner H, Briggs ADM, Brooks PM, Brown J, Brugha TS, Buchbinder R, Buckle GC, Bukhman G, Bulloch AG, Burch M, Burnett R, Cardenas R, Cabral NL, Campos-Nonato IR, Campuzano JC, Carapetis JR, Carpenter DO, Caso V, Castaneda-Orjuela CA, Catala-Lopez F, Chadha VK, Chang JC, Chen H, Chen W, Chiang PP, Chimed-Ochir O, Chowdhury R, Christensen H, Christophi CA, Chugh SS, Cirillo M, Coggeshall M, Cohen A, Colistro V, Colquhoun SM, Contreras AG, Cooper LT, Cooper C, Cooperrider K, Coresh J, Cortinovis M, Criqui MH, Crump JA, Cuevas-Nasu L, Dandona R, Dandona L, Dansereau E, Dantes HG, Dargan PI, Davey G, Davitoiu D V., Dayama A, De La Cruz-Gongora V, De La Vega SF, De Leo D, Del Pozo-Cruz B, Dellavalle RP, Deribe K, Derrett S, Des Jarlais DC,

Dessalegn M, DeVeber GA, Dharmaratne SD, Diaz-Torne C, Ding EL, Dokova K, Dorsey ER, Driscoll TR, Duber H, Durrani AM, Edmond KM, Ellenbogen RG, Endres M, Ermakov SP, Eshrati B, Esteghamati A, Estep K, Fahimi S, Farzadfar F, Fay DFJ, Felson DT, Fereshtehnejad SM, Fernandes JG, Ferri CP, Flaxman A, Foigt N, Foreman KJ, Fowkes FGR, Franklin RC, Furst T, Futran ND, Gabbe BJ, Gankpe FG, Garcia-Guerra FA, Geleijnse JM, Gessner BD, Gibney KB, Gillum RF, Ginawi IA, Giroud M, Giussani G, Goenka S, Goginashvili K, Gona P, De Cosio TG, Gosselin RA, Gotay CC, Goto A, Gouda HN, Guerrant RL, Gugnani HC, Gunnell D, Gupta R, Gupta R, Gutierrez RA, Hafezi-Nejad N, Hagan H, Halasa Y, Hamadeh RR, Hamavid H, Hammami M, Hankey GJ, Hao Y, Harb HL, Haro JM, Havmoeller R, Hay RJ, Hay S, Hedayati MT, Pi IBH, Heydarpour P, Hijar M, Hoek HW, Hoffman HJ, Hornberger JC, Hosgood HD, Hossain M, Hotez PJ, Hoy DG, Hsairi M, Hu H, Hu G, Huang JJ, Huang C, Huiart L, Husseini A, Iannarone M, Iburg KM, Innos K, Inoue M, Jacobsen KH, Jassal SK, Jeemon P, Jensen PN, Jha V, Jiang G, Jiang Y, Jonas JB, Joseph J, Juel K, Kan H, Karch A, Karimkhani C, Karthikeyan G, Katz R, Kaul A, Kawakami N, Kazi DS, Kemp AH, Kengne AP, Khader YS, Khalifa SEAH, Khan EA, Khan G, Khang YH, Khonelidze I, Kieling C, Kim D, Kim S, Kimokoti RW, Kinfu Y, Kinge JM, Kissela BM, Kivipelto M, Knibbs L, Knudsen AK, Kokubo Y, Kosen S, Kramer A, Kravchenko M, Krishnamurthi R V., Krishnaswami S, Defo BK, Bicer BK, Kuipers EJ, Kulkarni VS, Kumar K, Kumar GA, Kwan GF, Lai T, Lalloo R, Lam H, Lan Q, Lansingh VC, Larson H, Larsson A, Lawrynowicz AEB, Leasher JL, Lee JT, Leigh J, Leung R, Levi M, Li B, Li Y, Li Y, Liang J, Lim S, Lin HH, Lind M, Lindsay MP, Lipshultz SE, Liu S, Lloyd BK, Ohno SL, Logroscino G, Looker KJ, Lopez AD, Lopez-Olmedo N, Lortet-Tieulent J, Lotufo PA, Low N, Lucas RM, Lunevicius R, Lyons RA, Ma J, Ma S, MacKay MT, Majdan M, Malekzadeh R, Mapoma CC, Marcenes W, March LM, Margono C, Marks GB, Marzan MB, Masci JR, Mason-Jones AJ, Matzopoulos RG, Mayosi BM, Mazorodze TT, McGill NW, McGrath JJ, McKee M, McLain A, McMahon BJ, Meaney PA, Mehndiratta MM, Mejia-Rodriguez F, Mekonnen W, Melaku YA, Meltzer M, Memish ZA, Mensah G, Meretoja A, Mhimbira FA, Micha R, Miller TR, Mills EJ, Mitchell PB, Mock CN, Moffitt TE, Ibrahim NM, Mohammad KA, Mokdad AH,

Mola GL, Monasta L, Montico M, Montine TJ, Moore AR, Moran AE, Morawska L, Mori R, Moschandreas J, Moturi WN, Moyer M, Mozaffarian D, Mueller UO, Mukaigawara M, Murdoch ME, Murray J, Murthy KS, Naghavi P, Nahas Z, Naheed A, Naidoo KS, Naldi L, Nand D, Nangia V, Narayan KMV, Nash D, Nejjari C, Neupane SP, Newman LM, Newton CR, Ng M, Ngalesoni FN, Nhung NT, Nisar MI, Nolte S, Norheim OF, Norman RE, Norrving B, Nyakarahuka L, Oh IH, Ohkubo T, Omer SB, Opio JN, Ortiz A, Pandian JD, Panelo CIA, Papachristou C, Park EK, Parry CD, Caicedo AJP, Patten SB, Paul VK, Pavlin BI, Pearce N, Pedraza LS, Pellegrini CA, Pereira DM, Perez-Ruiz FP, Perico N, Pervaiz A, Pesudovs K, Peterson CB, Petzold M, Phillips MR, Phillips D, Phillips B, Piel FB, Plass D, Poenaru D, Polanczyk G V., Polinder S, Pope CA, Popova S, Poulton RG, Pourmalek F, Prabhakaran D, Prasad NM, Qato D, Quistberg DA, Rafay A, Rahimi K, Rahimi-Movaghar V, Rahman SU, Raju M, Rakovac I, Rana SM, Razavi H, Refaat A, Rehm J, Remuzzi G, Resnikoff S, Ribeiro AL, Riccio PM, Richardson L, Richardus JH, Riederer AM, Robinson M, Roca A, Rodriguez A, Rojas-Rueda D, Ronfani L, Rothenbacher D, Roy N, Ruhago GM, Sabin N, Sacco RL, Ksoreide K, Saha S, Sahathevan R, Sahraian MA, Sampson U, Sanabria JR, Sanchez-Riera L, Santos IS, Satpathy M, Saunders JE, Sawhney M, Saylan MI, Scarborough P, Schoettker B, Schneider IJC, Schwebel DC, Scott JG, Seedat S, Sepanlou SG, Serdar B, Servan-Mori EE, Shackelford K, Shaheen A, Shahraz S, Levy TS, Shangguan S, She J, Sheikhbahaei S, Shepard DS, Shi P, Shibuya K, Shinohara Y, Shiri R, Shishani K, Shiue I, Shrime MG, Sigfusdottir ID, Silberberg DH, Simard EP, Sindi S, Singh JA, Singh L, Skirbekk V, Sliwa K, Soljak M, Soneji S, Soshnikov SS, Speyer P, Sposato LA, Sreeramareddy CT, Stoeckl H, Stathopoulou VK, Steckling N, Stein MB, Stein DJ, Steiner TJ, Stewart A, Stork E, Stovner LJ, Stroumpoulis K, Sturua L, Sunguya BF, Swaroop M, Sykes BL, Tabb KM, Takahashi K, Tan F, Tandon N, Tanne D, Tanner M, Tavakkoli M, Taylor HR, Te Ao BJ, Temesgen AM, Have M Ten, Tenkorang EY, Terkawi AS, Theadom AM, Thomas E, Thorne-Lyman AL, Thrift AG, Tleyjeh IM, Tonelli M, Topouzis F, Towbin JA, Toyoshima H, Traebert J, Tran BX, Trasande L, Trillini M, Truelsen T, Trujillo U, Tsilimbaris M, Tuzcu EM, Ukwaja KN, Undurraga EA, Uzun SB, Van Brakel WH, Van De Vijver S, Dingenen R Van, Van Gool CH,

Varakin YY, Vasankari TJ, Vavilala MS, Veerman LJ, Velasquez-Melendez G, Venketasubramanian N, Vijayakumar L, Villalpando S, Violante FS, Vlassov V V., Waller S, Wallin MT, Wan X, Wang L, Wang J, Wang Y, Warouw TS, Weichenthal S, Weiderpass E, Weintraub RG, Werdecker A, Wessells KR, Westerman R, Wilkinson JD, Williams HC, Williams TN, Woldeyohannes SM, Wolfe CDA, Wong JQ, Wong H, Woolf AD, Wright JL, Wurtz B, Xu G, Yang G, Yano Y, Yenesew MA, Yentur GK, Yip P, Yonemoto N, Yoon SJ, Younis M, Yu C, Kim KY, Zaki MES, Zhang Y, Zhao Z, Zhao Y, Zhu J, Zonies D, Zunt JR, Salomon JA, Murray CJL. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386(9995):743–800.

53. Adams MA. Biomechanics of back pain. Acupunct Med. 2004 Dec;22(4):178–188.

54. Hoy D, Brooks P, Blyth F, Buchbinder R. The Epidemiology of low back pain. Best Pract Res Clin Rheumatol. 2010 Dec;24(6):769–781.

55. Polatin PB, Kinney RK, Gatchel RJ, Lillo E, Mayer TG. Psychiatric illness and chronic low-back pain. The mind and the spine--which goes first? Spine (Phila Pa 1976). 1993 Jan;18(1):66–71.

56. Rudert M, Tillmann B. Lymph and blood supply of the human intervertebral disc. Cadaver study of correlations to discitis. Acta Orthop Scand. 1993 Feb;64(1):37–40.

57. Zhao CQ, Wang LM, Jiang LS, Dai LY. The cell biology of intervertebral disc aging and degeneration. Ageing Res Rev. 2007;6(3):247–261.

58. Pazzaglia, U.E., Salisbury, J.R., Byers PD. Development and involution of the notochord in the human spine. J R Soc Med. 1989;82(7):413–415.

59. Le Maitre CL, Freemont AJ, Hoyland JA. Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration. Arthritis Res Ther. 2007;9(3):R45.

60. Zhang GZ, Deng YJ, Xie QQ, Ren EH, Ma ZJ, He XG, Gao YC, Kang XW. Sirtuins and intervertebral disc degeneration: Roles in inflammation, oxidative stress, and mitochondrial function. Clin Chim Acta. 2020;508(March):33–42.

61. Rajasekaran S, Venkatadass K, Naresh Babu J, Ganesh K, Shetty AP. Pharmacological enhancement of disc diffusion and differentiation of healthy, ageing and degenerated discs: Results from in-vivo serial post-contrast MRI studies in 365 human lumbar discs. Eur Spine J. 2008;17(5):626–643.

62. Fields AJ, Ballatori A, Liebenberg EC, Lotz JC. Contribution of the endplates to disc degeneration. Curr Mol Biol reports. 2018 Dec;4(4):151–160.

63. Benoist M. Natural history of the aging spine. Eur Spine J. 2003;12(SUPPL. 2):86–89.

64. Cavanaugh JM, Ozaktay AC, Yamashita T, Avramov A, Getchell T V, King AI. Mechanisms of low back pain: a neurophysiologic and neuroanatomic study. Clin Orthop Relat Res. 1997 Feb;(335):166–180.

65. Rosen CJ, Bouxsein ML. Mechanisms of disease: is osteoporosis the obesity of bone? Nat Clin Pract Rheumatol. 2006 Jan;2(1):35–43.

66. Bradley WG. Use of contrast in MR imaging of the lumbar spine. Magn Reson Imaging Clin N Am. 1999 Aug;7(3):439–457, vii.

67. RA D, Rainville J, DL K. What can the history and physical examination tell us about low back pain? JAMA. 1992 Aug 12;268(6):760–765.

68. Lee BH, Moon SH, Suk KS, Kim HS, Yang JH, Lee HM. Lumbar Spinal Stenosis: Pathophysiology and Treatment Principle: A Narrative Review. Asian Spine J. 2020 Oct;14(5):682–693.

69. Colak A, Topuz K, Kutlay M, Kaya S, Simşek H, Cetinkal A, Demircan MN. A less invasive surgical approach in the lumbar lateral recess stenosis: direct approach to the medial wall of the pedicle. Eur spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc. 2008 Dec;17(12):1745–1751.

70. Videman T, Saarela J, Kaprio J, Näkki A, Levälahti E, Gill K, Peltonen L, Battié MC. Associations of 25 structural, degradative, and inflammatory candidate genes with lumbar disc desiccation, bulging, and height narrowing. Arthritis Rheum. 2009;60(2):470–481.

71. De Smet a. a. Fundamentals of Diagnostic Radiology. 4th editio. William E. Brant, MD, FACR; Clyde A. Helms M, editor. Vol. 278, Jama. 2012. 525–525 p.

72. Williams AL, Murtagh FR, Rothman SLG, Sze GK. Lumbar disc nomenclature: Version 2.0. Am J Neuroradiol. 2014;35(11):2029.

73. Fardon DF. Nomenclature and classification of lumbar disc pathology. Spine (Phila Pa 1976). 2001;26(5):461–462.

74. Zhu F, Bao H, Yan P, Liu S, Bao M, Zhu Z, Liu Z, Qiu Y. Do the disc degeneration and osteophyte contribute to the curve rigidity of degenerative scoliosis? BMC Musculoskelet Disord. 2017 Mar;18(1):128.

75. Teichtahl AJ, Urquhart DM, Wang Y, Wluka AE, Heritier S, Cicuttini FM. A Dose-response relationship between severity of disc degeneration and intervertebral disc height in the lumbosacral spine. Arthritis Res Ther. 2015 Oct;17:297.

76. Rajasekaran S, Kanna RM, Reddy RR, Natesan S, Raveendran M, Cheung KMC, Chan D, Kao PYP, Yee A, Shetty AP. How Reliable Are the Reported Genetic Associations in Disc Degeneration?: The Influence of Phenotypes, Age, Population Size, and Inclusion Sequence in 809 Patients. Spine (Phila Pa 1976). 2016 Nov;41(21):1649–1660.

77. Pákó J, Kunos L, Mészáros M, Tárnoki DL, Tárnoki ÁD, Horváth I, Bikov A. Decreased Levels of Anti-Aging Klotho in Obstructive Sleep Apnea. Rejuvenation Res. 2020;23(3):256–261.

78. Kim JH, Hwang KH, Park KS, Kong ID, Cha SK. Biological Role of Anti-aging Protein Klotho. J lifestyle Med. 2015 Mar;5(1):1–6.

79. Moreno JA, Izquierdo MC, Sanchez-Niño MD, Suárez-Alvarez B, Lopez-Larrea C, Jakubowski A, Blanco J, Ramirez R, Selgas R, Ruiz-Ortega M, Egido J, Ortiz

A, Sanz AB. The inflammatory cytokines TWEAK and TNFα reduce renal klotho expression through NFκB. J Am Soc Nephrol. 2011 Jul;22(7):1315–1325.

80. Komaba H, Fukagawa M. Vitamin D and secreted Klotho: a long-awaited panacea for vascular calcification? Kidney Int. 2012 Dec 2;82(12):1248–1250.

81. Kawano K ichi, Ogata N, Chiano M, Molloy H, Kleyn P, Spector TD, Uchida M, Hosoi T, Suzuki T, Orimo H, Inoue S, Nabeshima Y, Nakamura K, Kuro-o M, Kawaguchi H. Klotho gene polymorphisms associated with bone density of aged postmenopausal women. J bone Miner Res Off J Am Soc Bone Miner Res. 2002 Oct;17(10):1744–1751.

82. Ogata N, Matsumura Y, Shiraki M, Kawano K, Koshizuka Y, Hosoi T, Nakamura K, Kuro-O M, Kawaguchi H. Association of klotho gene polymorphism with bone density and spondylosis of the lumbar spine in postmenopausal women. Bone. 2002 Jul;31(1):37–42.

83. Bi F, Liu W, Wu Z, Ji C, Chang C. Antiaging Factor Klotho Retards the Progress of Intervertebral Disc Degeneration through the Toll-Like Receptor 4-NF- κ B Pathway. Int J Cell Biol. 2020;2020:9–11.

84. Kyvik KO, Green A, Beck-Nielsen H. The new Danish Twin Register: establishment and analysis of twinning rates. Int J Epidemiol. 1995 Jun;24(3):589–596.

85. Kushida CA, Littner MR, Morgenthaler T, Alessi CA, Bailey D, Coleman JJ, Friedman L, Hirshkowitz M, Kapen S, Kramer M, Lee-Chiong T, Loube DL, Owens J, Pancer JP, Wise M. Practice parameters for the indications for polysomnography and related procedures: an update for 2005. Sleep. 2005 Apr;28(4):499–521.

86. Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, Marcus CL, Mehra R, Parthasarathy S, Quan SF, Redline S, Strohl KP, Davidson Ward SL, Tangredi MM. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. J Clin sleep Med JCSM Off Publ Am Acad Sleep Med. 2012

Oct;8(5):597–619.

87. Szily M, Tarnoki AD, Tarnoki DL, Kovacs DT, Forgo B, Lee J, Kim E, Sung J, Kunos L, Meszaros M, Muller V, Bikov A. Genetic influences on the onset of obstructive sleep apnoea and daytime sleepiness: A twin study. Respir Res. 2019;20(1).

88. Tarnoki AD, Tarnoki DL, Oláh C, Szily M, Kovacs DT, Dienes A, Piroska M, Forgo B, Pinheiro M, Ferreira P, Kostyál L, Meszaros M, Pako J, Kunos L, Bikov A. Lumbar spine abnormalities in patients with obstructive sleep apnoea. Sci Rep. 2021 Aug;11(1):16233.

89. Fardon DF, Williams AL, Dohring EJ, Murtagh FR, Gabriel Rothman SL, Sze GK. Lumbar disc nomenclature: version 2.0: Recommendations of the combined task forces of the North American Spine Society, the American Society of Spine Radiology and the American Society of Neuroradiology. Spine J. 2014 Nov;14(11):2525–2545.

90. Farshad-Amacker NA, Farshad M, Winklehner A, Andreisek G. MR imaging of degenerative disc disease. Eur J Radiol. 2015 Sep;84(9):1768–1776.

91. Videman T, Battié MC, Ripatti S, Gill K, Manninen H, Kaprio J. Determinants of the progression in lumbar degeneration: a 5-year follow-up study of adult male monozygotic twins. Spine (Phila Pa 1976). 2006 Mar;31(6):671–678.

92. Pfirrmann CW, Metzdorf A, Zanetti M, Hodler J, Boos N. Magnetic resonance classification of lumbar intervertebral disc degeneration. Spine (Phila Pa 1976). 2001 Sep;26(17):1873–1878.

93. Blangero J, Diego VP, Dyer TD, Almeida M, Peralta J, Kent JWJ, Williams JT, Almasy L, Göring HHH. A kernel of truth: statistical advances in polygenic variance component models for complex human pedigrees. Adv Genet. 2013;81:1–31.

94. Schwab RJ, Pasirstein M, Kaplan L, Pierson R, Mackley A, Hachadoorian R, Arens R, Maislin G, Pack AI. Family Aggregation of Upper Airway Soft Tissue Structures in Normal Subjects and Patients with Sleep Apnea. Vol. 173, American

Journal of Respiratory and Critical Care Medicine. 2006. p. 453–463.

95. Tarnoki AD, Tarnoki DL, Stazi MA, Medda E, Cotichini R, Nisticò L, Fagnani C, Lucatelli P, Boatta E, Zini C, Fanelli F, Baracchini C, Meneghetti G, Osztovits J, Jermendy G, Préda I, Kiss RG, Metneki J, Horvath T, Karlinger K, Racz A, Lannert A, Molnar AA, Littvay L, Garami Z, Berczi V, Schillaci G. Heritability of central blood pressure and arterial stiffness: a twin study. J Hypertens. 2012 Aug;30(8):1564–1571.

96. Tarnoki DL, Tarnoki AD, Littvay L, Lazar Z, Karlinger K, Molnar AA, Melicher D, Garami Z, Berczi V, Horvath I. Transmission of second-hand smoke sensitivity and smoking attitude in a family. Ann Agric Environ Med. 2014;21(4):771–775.

97. Tarnoki AD, Tarnoki DL, Medda E, Cotichini R, Stazi MA, Fagnani C, NisticÃ L, Lucatelli P, Boatta E, Zini C, Fanelli F, Baracchini C, Meneghetti G, Schillaci G, Osztovits J, Jermendy G, Kiss RBG, PrÃ da IN, Karlinger K, Lannert A, Metneki J, Molnar AA, Garami Z, Berczi V, Halasz I, Baffy G. Bioimpedance analysis of body composition in an international twin cohort. Obes Res Clin Pract. 2014;8(3):e201-98.

98. Khurana S, Sharda S, Saha B, Kumar S, Guleria R, Bose S. Canvassing the aetiology, prognosis and molecular signatures of obstructive sleep apnoea. Biomarkers Biochem Indic Expo response, susceptibility to Chem. 2019 Feb;24(1):1–16.

99. Qureshi IA, Mehler MF. Epigenetics of sleep and chronobiology. Curr Neurol Neurosci Rep. 2014 Mar;14(3):432.

100. Williams NJ, Jean-Louis G, Ravenell J, Seixas A, Islam N, Trinh-Shevrin C, Ogedegbe G. A community-oriented framework to increase screening and treatment of obstructive sleep apnea among blacks. Sleep Med. 2016 Feb;18:82–87.

101. Glazer SA, Erickson AL, Crosby RD, Kieda J, Zawisza A, Deitel M. The Evaluation of Screening Questionnaires for Obstructive Sleep Apnea to Identify High-Risk Obese Patients Undergoing Bariatric Surgery. Obes Surg. 2018 Nov;28(11):3544–3552.

102. Westlake K, Dostalova V, Plihalova A, Pretl M, Polak J. The Clinical Impact of Systematic Screening for Obstructive Sleep Apnea in a Type 2 Diabetes Population-Adherence to the Screening-Diagnostic Process and the Acceptance and Adherence to the CPAP Therapy Compared to Regular Sleep Clinic Patients. Front Endocrinol (Lausanne). 2018;9:714.

103. Drager LF, Togeiro SM, Polotsky VY, Lorenzi-Filho G. Obstructive sleep apnea: a cardiometabolic risk in obesity and the metabolic syndrome. J Am Coll Cardiol. 2013 Aug;62(7):569–576.

104. Pahkala R, Seppä J, Ikonen A, Smirnov G, Tuomilehto H. The impact of pharyngeal fat tissue on the pathogenesis of obstructive sleep apnea. Sleep Breath. 2014 May 23;18(2):275–282.

105. de Carvalho MMB, Coutinho RQ, Barros IML, Costa LOBF, Medeiros AKL, Lustosa TC, Medeiros CA, França MV, Couto TLG, Montarroyos UR, Somers VK, Pedrosa RP. Prevalence of Obstructive Sleep Apnea and Obesity Among Middle-Aged Women: Implications for Exercise Capacity. J Clin sleep Med JCSM Off Publ Am Acad Sleep Med. 2018 Sep;14(9):1471–1475.

106. Hargens TA, Martin RA, Strosnider CL, Giersch GEW, Womack CJ. Obstructive sleep apnea negatively impacts objectively measured physical activity. Sleep Breath. 2019 Jun;23(2):447–454.

107. MacLean JE, DeHaan K, Chowdhury T, Nehme J, Bendiak GN, Hoey L, Horwood L, Pasterkamp H, Kirk V, Constantin E, Katz SL. The scope of sleep problems in Canadian children and adolescents with obesity. Sleep Med. 2018 Jul;47:44–50.

108. Ng SSS, Liu EKH, Ma RCW, Chan TO, To KW, Chan KKP, Ngai J, Yip WH, Ko FWS, Wong CK, Hui DSC. Effects of CPAP therapy on visceral fat thickness, carotid intima-media thickness and adipokines in patients with obstructive sleep apnoea. Respirology. 2017 May;22(4):786–792.

109. Brożyna-Tkaczyk K, Myśliński W, Mosiewicz J. The Assessment of Endothelial Dysfunction among OSA Patients after CPAP Treatment. Medicina (Kaunas). 2021 Mar;57(4).

110. Sharma SK, Agrawal S, Damodaran D, Sreenivas V, Kadhiravan T, Lakshmy R, Jagia P, Kumar A. CPAP for the metabolic syndrome in patients with obstructive sleep apnea. N Engl J Med. 2011 Dec;365(24):2277–2286.

111. Eyigor H, Selcuk OT, Osma U, Koca R, Yilmaz MD. Cervical osteophytes: a rare cause of obstructive sleep apnea. J Craniofac Surg. 2012 Sep;23(5):e444-6.

112. Perry JC, Guindalini C, Bittencourt L, Garbuio S, Mazzotti DR, Tufik S. Whole blood hypoxia-related gene expression reveals novel pathways to obstructive sleep apnea in humans. Respir Physiol Neurobiol. 2013 Dec;189(3):649–654.

113. Oichi T, Taniguchi Y, Oshima Y, Tanaka S, Saito T. Pathomechanism of intervertebral disc degeneration. JOR spine. 2020 Mar;3(1):e1076.

114. Gottlieb DJ, Punjabi NM. Diagnosis and Management of Obstructive Sleep Apnea: A Review. JAMA. 2020 Apr;323(14):1389–1400.

115. Samartzis D, Karppinen J, Chan D, Luk KDK, Cheung KMC. The association of lumbar intervertebral disc degeneration on magnetic resonance imaging with body mass index in overweight and obese adults: a population-based study. Arthritis Rheum. 2012 May;64(5):1488–1496.

116. Cai XY, Xia Y, Yang SH, Liu XZ, Shao ZW, Liu YL, Yang W, Xiong LM. Ropivacaine- and bupivacaine-induced death of rabbit annulus fibrosus cells in vitro: involvement of the mitochondrial apoptotic pathway. Osteoarthr Cartil. 2015 Oct;23(10):1763–1775.

117. Scharf B, Clement CC, Yodmuang S, Urbanska AM, Suadicani SO, Aphkhazava D, Thi MM, Perino G, Hardin JA, Cobelli N, Vunjak-Novakovic G, Santambrogio L. Age-related carbonylation of fibrocartilage structural proteins drives tissue degenerative modification. Chem Biol. 2013 Jul;20(7):922–934.

118. Pokharna HK, Phillips FM. Collagen crosslinks in human lumbar intervertebral disc aging. Spine (Phila Pa 1976). 1998 Aug;23(15):1645–1648.

119. Feng C, Yang M, Lan M, Liu C, Zhang Y, Huang B, Liu H, Zhou Y. ROS: Crucial Intermediators in the Pathogenesis of Intervertebral Disc Degeneration. Oxid Med Cell Longev. 2017;2017:5601593.

120. Molinos M, Almeida CR, Caldeira J, Cunha C, Gonçalves RM, Barbosa MA. Erratum: Inflammation in intervertebral disc degeneration and regeneration (Journal of the Royal Society Interface (2015) 12 (20141191) DOI:10.1098/rsif.2014.1191). Vol. 12, Journal of the Royal Society Interface. 2015. p. 20150429.

121. Li SA, Watanabe M, Yamada H, Nagai A, Kinuta M, Takei K. Immunohistochemical localization of Klotho protein in brain, kidney, and reproductive organs of mice. Cell Struct Funct. 2004 Dec;29(4):91–99.

122. Hiyama A, Arai F, Sakai D, Yokoyama K, Mochida J. The effects of oxygen tension and antiaging factor Klotho on Wnt signaling in nucleus pulposus cells. Arthritis Res Ther. 2012 May;14(3):R105.

123. Li JJ, Appleton SL, Gill TK, Vakulin A, Wittert GA, Antic NA, Taylor AW, Adams RJ, Hill CL. Association of Musculoskeletal Joint Pain With Obstructive Sleep Apnea, Daytime Sleepiness, and Poor Sleep Quality in Men. Arthritis Care Res (Hoboken). 2017 May;69(5):742–747.

10 Bibliography of own publications

10.1 Publications related to the current PhD thesis

Szily M, Tarnoki AD, Tarnoki DL, Kovacs DT, Forgo B, Lee J, et al. Genetic influences on the onset of obstructive sleep apnoea and daytime sleepiness: A twin study. Respiratory Research. 2019;20(1). **(IF: 3.924)**

Tarnoki AD, Tarnoki DL, Oláh C, Szily M, Kovacs DT, Dienes A, et al. Lumbar spine abnormalities in patients with obstructive sleep apnoea. Scientific Reports. 2021;11(1). **(IF:4.997)**

In total **IF: 8,921**

10.2 Publications not related to the current PhD thesis

Fekete M, Piroska M, Szily M, Erdei M, Jokkel Z, Szabo H, et al. Heritability analysis of liver stiffness detected by ultrasound shear wave elastography: A twin study. European Journal of Gastroenterology & Hepatology. 2021;33(1S). **(IF:2.586)**

Jokkel Z, Szily M, Sipos B, Oluk E, Piroska M, Kalina I, et al. The heritability of upper airway dimensions using MRI scans in twins. Applied Sciences. 2022;12(15):7646. **(IF: 2,838)**

Bikov A, Szabo H, Piroska M, Kunos L, Szily M, Ligeti B, et al. Gut microbiome in patients with obstructive sleep apnoea. Applied Sciences. 2022;12(4):2007. **(IF: 2,838)**

Hernyes A, Fejér B, Szabó H, Szalontai L, Persely A, Fekete M, Szily M, Jokkel Z, Debreceni R, Gyulai K et al. Az arteria carotis communis intima-media komplex sajátosságainak vizsgálata ultrahanggal - Technikai megfontolások és korrelációk egyéb atherosclerosisra utaló változókkal. Magyar Radiológia Online, 2019/3.

Fekete M, Erdei M, Dienes A, Persely A, Szily M, Bitai Z, Hernyes A, Stazi MA, Medda E, Fagnani C et al. A pajzsmirigy ultrahang-elasztográfiás vizsgálata ikreken Magyar Radiológia Online 2019; 10(1): 5/1-10

IF in total:18.342

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12 Supplements

12.1 Supplement 1: Epworth Sleepiness Scale

The Epworth Sleepiness Scale

The Epworth Sleepiness Scale is widely used in the field of sleep medicine as a subjective measure of apatient's sleepiness. The test is a list of eight situations in which you rate your tendency to become sleepy on a scale of 0, no chance of dozing, to 3, high chance of dozing. When you finish the test, add up the values of your responses. Your total score is based on a scale of 0 to 24. The scale estimates whether you are experiencing excessive sleepiness that possibly requires medical attention.

How Sleepy Are You?

How likely are you to doze off or fall asleep in the following situations? You should rate your chancesof dozing off, not just feeling tired. Even if you have not done some of these things recently try to determine how they would have affected you. For each situation, decide whether or not you would have:

- No chance of dozing =0
- Slight chance of dozing =1
- Moderate chance of dozing =2
- High chance of dozing =3

Write down the number corresponding to your choice in the right hand column. Total your score below.

Situation	Chance of Dozing
Sitting and reading	□
Watching TV	□
Sitting inactive in a public place (e.g., a theater ora meeting)	□
As a passenger in a car for an hour without a break	□
Lying down to rest in the afternoon when circumstances permit	□
Sitting and talking to someone	□
Sitting quietly after a lunch without alcohol	□
In a car, while stopped for a few minutes in traffic	□

Total Score = _____

Analyze Your Score

Interpretation:

0-7: It is unlikely that you are abnormally sleepy.

8-9: You have an average amount of daytime sleepiness.

10-15: You may be excessively sleepy depending on the situation. You may want to considerseeking medical attention.

16-24: You are excessively sleepy and should consider seeking medical attention.

Reference: Johns MW. A new method for measuring daytime sleepiness: The Epworth Sleepiness Scale. *Sleep*

1991; 14(6):540-5.

12.2 Supplement 2: 24-item Roland-Morris Disability Questionnaire

24-item Roland-Morris Disability Questionnaire

- □ I stay at home most of the time because of my back.
- □ I change position frequently to try and get my back comfortable.
- □ I walk more slowly than usual because of my back.

- □ Because of my back I am not doing any of the jobs that I usually do around the house.
- □ Because of my back, I use a handrail to get upstairs.
- □ Because of my back, I lie down to rest more often.
- □ Because of my back, I have to hold on to something to get out of an easy chair.
- □ Because of my back, I try to get other people to do things for me.
- □ I get dressed more slowly than usual because of my back.
- □ I only stand for short periods of time because of my back.
- □ Because of my back, I try not to bend or kneel down.
- □ I find it difficult to get out of a chair because of my back.
- □ My back is painful almost all the time.
- □ I find it difficult to turn over in bed because of my back.
- □ My appetite is not very good because of my back pain.
- □ I have trouble putting on my socks (or stockings) because of the pain in my back.
- □ I only walk short distances because of my back.
- □ I sleep less well because of my back.
- □ Because of my back pain, I get dressed with help from someone else.
- □ I sit down for most of the day because of my back.
- □ I avoid heavy jobs around the house because of my back.
- □ Because of my back pain, I am more irritable and bad tempered with people than usual.
- □ Because of my back, I go upstairs more slowly than usual.
- □ I stay in bed most of the time because of my back.

Score:_____

12.3 Suplement 3 : Low Back Pain Questionnaire

Questionnaire about lower back pain

a.1. Have you ever had lower back pain?

Yes ☐ No ☐

a.2. Does lower back pain limit your normal daily activities or change your daily routine more than one day?

Yes ☐ No ☐

a.3. When was the last time when you had lower back pain?

In the last 2 years ☐ Last year ☐ In the last 6 months ☐ In the last 4 weeks ☐

a.4. Did you have lower back pain in the last 4 weeks? (Except for lower back pain due to infection with high temperature or menstruation)

Yes ☐ No ☐

a.5. If yes, did this lower back pain limit your normal daily activities or change your daily routine more than one day?

Yes ☐ No ☐

a.6. How long did this pain take?

Less than 3 months ☐ More than 3 months, but less than 7 months ☐

7 months or more, but less than 3 years ☐ 3 years or more ☐

a.7. Please sign (with X in the table below), how was the intensity of your pain on a 0-10 scale, where 0 means "No pain" and 10 means "Worst possible pain":

… the intensity of the last lower back pain:

0	1	2	3	4	5	6	7	8	9	10
No pain										Worst possible pain

… the intensity of the worst lower back pain:

0	1	2	3	4	5	6	7	8	9	10
No pain										Worst possible pain

a.8. If you had lower back pain in the last 4 weeks, how often did you have this pain?

Just some days ☐ Most of the days ☐ Everyday ☐

a.9. How many different lower back pain episodes did you have in your life that were strong enough to pain limit your normal daily activities or change your daily routine more than one day? *(Different episode means that at least a month passed between the two episodes when you had no pain.)*

How many times? _____

a.10. Were you unable to work or did you reduce your normal daily activities due to lower back pain in the last 12 months?

Yes ☐ No ☐

a.11. If yes, please estimate the total number of days when you dropped out of your job:

0-30 days ☐ 30-60 days ☐ 60-90 days ☐ More than 90 days ☐

Note about plagiarism in PhD thesis

Some passages from Methods, Result and Discussion chapters have been quoted verbatim from the following sources:

Szily M, Tarnoki AD, Tarnoki DL, Kovacs DT, Forgo B, Lee J, et al. Genetic influences on the onset of obstructive sleep apnoea and daytime sleepiness: A twin study. Respir Res. 2019;20(1).

Tarnoki AD, Tarnoki DL, Oláh C, Szily M, Kovacs DT, Dienes A, et al. Lumbar spine abnormalities in patients with obstructive sleep apnoea. Sci Rep. 2021 Dec 1;11(1).