GENETICS OF OBSTRUCTIVE SLEEP APNEA

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Thesis for the Degree of Master of Science
University of Iceland
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School of Health Sciences

HÁSKÓLI ÍSLANDS

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Hafdís Þórunn Helgadóttir

Ritgerð til meistaragráðu í líf- og læknavísindum

Umsjónarkennari: Ingileif Jónsdóttir

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Ágrip

Kæfisvefn er algengur og margþættur sjúkdómur og orsakaþættir margir. Hann einkennist af öndunarstoppum í svefni sem eru vegna þrengsla í efri öndunarvegi. Kæfisvefn er algengari hjá körlum en konum og algengastur hjá miðaldra fólki. Fyrri rannsóknir á kæfisvefni hafa sýnt fram á ættlægni sjúkdómsins en engir erfðabreytileikar hafa fundist sem tengjast sjúkdómnum.

Tilgangur þessarar rannsóknar var að athuga ættlægni kæfisvefns á Íslandi, að finna litningasvæði eða gen sem tengjast kæfisvefn, athuga áhrif erfðabreytileika sem tengjast offitu á áhættu á kæfisvefni og skoða áhrif erfðabreytileika í genum sem gætu tengst kæfisvefni. Dulkóðaður ættfræðigrunnur Íslendingabókar var fyrir rannsókn á ættlægni. Tengslagreining á öllu erfðamengi í íslenskum kæfisvefnssjúklingum og fjölskyldum þeirra var gerð til að leita að tengslum við litningasvæði, og fylgnirannsókn á völdum erfðabreytileikum gerð í sjúklingum og samanburðarhópi.

Við sýndum fram á að ættingjar kæfisvefnssjúklinga eru líklegri til að fá kæfisvefn heldur en Íslendingar almennt. Fyrstu gráðu ættingi hefur meira en tvöfalda hlutfallsáhættu (RR=2.33). Offita eykur áhættuna á kæfisvefn, sem er þreföld meðal fyrstu gráðu ættingja kæfisvefnssjúklinga sem hafa líkamsþyngdarstuðul (e. BMI) 30 og hærra.

Tengslagreining sýndi ekki marktæk tengsl milli ákveðinna litningasvæða og þeirra svipgerða kæfisvefns sem athugaðar voru. Til að reyna að þrengja þau svæði sem sýndu hugsanleg tengsl við kæfisvefn var fylgni einbasabreytileika (e. SNP) á 10 svæðum með tengslastuðul (e. LOD score) yfir 1.5 við viðkomandi svipgerð skoðuð. Einn erfðabreytileiki á 11p13 sýndi marktæka fylgni ($P=1.26x10^{-6}$, OR=1.30 við kæfisvefn meðal sjúklínga með offitu). Þegar þessi breytileiki var athugaður í fleiri íslenskum sjúklingum og bandarískum var fylgnin ekki lengur tölfræðilega marktæk.

32 erfðabreytileikar sem tengdir eru viðoffitu voru athugaðir í íslenskum kæfisvefnssjúklingum og tveir þeirra sýndu marktæka fylgni við kæfisvefn. Einbasabreytileiki í FTO geninu á 16q12 jók hættu á kæfisvefni (P=0.0009, OR=1.096), en fylgnin hvarf þegar leiðrétt var fyrir líkamsþyngdarstuðli og kyni (P=0.76). Annar einbasabreytileiki, rs10838738, í MTCH2 geninu á 11p11 sýndi fylgni við kæfisvefn (P=0.015, OR=0.93) sem styrktist þegar leiðrétt var fyrir kyni og líkamsþyngdarstuðli (P=8.5×10-5, OR=0.879). Ólíkt fylgni þessa MTCH2 breytleika við aukinn líkamsþyngdarstuðul hafði hann vernandi áhrif á kæfisvefn. Fylgnin var staðfest í áströlskum kæfisvefnssjúklingum (*P*=0.044, OR=0.908 eftir leiðréttingu fyrir kyni og líkamsþyngdarstuðli).

Í stuttu máli sýndu niðurstöður rannsóknarinnar fram á ættlægni kæfisvefns og að hár líkamsþyngdarstuðull eykur hana. Engin marktæk tengsl kæfisvefns við litningasvæði eða gen fundust í tengslagreiningu en frekari rannsókn á svæðum sem sýndu hugsamnleg tengsl sýndi fram á fylgni eins erfðabreytileika við kæfisvefn, sem var hvorki staðfest í öðru útaki Íslendinga né Bandaríkjamanna. Erfðabreytileikar tengdir offitu reyndust ekki auka áhættu á kæfisvefn, fyrir utan breytileika í *FTO* geninu sem virðist hafa áhrif á kæfisvefn gegnum boðleiðir tengdar líkamsþyngd, og breytileika í *MTCH2* geninu sem dregur úr líkum á kæfisvefni að því er virðist gegnum aðrar boðleiðir.

Abstract

Obstructive sleep apnea (OSA) is a common complex trait with many potential contributing factors. It is characterized by pauses in breathing during sleep due to obstruction in the upper airway. OSA is more common in men than women and in the middle-aged population. Previous studies have showed familial aggregation, but no significant association of sequence variants with OSA has been reported.

The aims of the study were to assess familial aggregation of OSA in Iceland, to identify chromosomal regions/loci and genes that are linked with OSA, to analyse the effects of obesity-linked variants on the risk of OSA and to validate sequence variants in genes with suggestive association with OSA. The approaches used include analysis of familiality using a nationwide genealogy database, whole genome linkage scan in Icelandic OSA families and case-control association analyses of selected sequence variants.

We showed that relatives of OSA patients are more likely to have OSA than individuals in the general population, where the first-degree relatives have more than a twofold relative risk (RR for all OSA=2.33). It was also shown that obesity adds to the risk of OSA, which was threefold for first-degree relatives of obese OSA patients.

No genome wide significant linkage (a LOD score >3.7) to any chromosomal regions was observed for the OSA phenotypes tested. To finemap the suggestive linkage regions, SNPs under 10 linkage peaks with LOD scores ≥1.5 were tested for association in the corresponding OSA phenotypes and a significant association of one variant at 11p13 (P=1.26x10^{-6}, OR=1.30 for obese OSA patients with severe, moderate or mild disease) was observed. However, when tested in additional OSA samples from Iceland and the USA the association was not replicated.

Various previously published variants known to associate with obesity-related traits were tested for association with OSA in Iceland and two significant associations were found. Firstly, a variant in the *FTO* gene at 16q12 associated with OSA (P=0.0009, OR=1.096), but when adjusted for BMI and gender the association disappeared (P=0.76). Secondly, an obesity-related variant, rs10838738, in the *MTCH2* gene at 11p11 associated with OSA (P=0.015, OR=0.93). This signal was strengthened after adjusting for gender and BMI (P=8.5×10^{-5}, OR=0.879). Interestingly, the allele of the *MTCH2* variant that associates with increase in BMI was found to associate with reduced risk of OSA. This association was replicated in an Australian dataset (P=0.044, OR=0.908 after adjusting for BMI and gender).

Taken together, OSA was shown to aggregate in families and high BMI increases the familial risk. Linkage analysis did not reveal any significant associations with OSA and finemapping of suggestive regions showed one significant association that did not replicate in other sample sets. Known obesity-related variants were not found to contribute to the risk of OSA except for a variant in the *FTO* gene that conferred risk through BMI related pathways and a variant in the MTCH2 gene that reduced the risk of OSA, not through obesity.

Further studies based on genome-wide association and whole genome sequencing will hopefully reveal sequence variants that confer risk of OSA and help us to understand the mechanisms involved.

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First of all I thank Ingileif Jónsdóttir for all the support with this project. Your positive attitude encouraged me to continue with this project. Whenever I have needed help you have been ready to assist and push me forward.

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Abbreviations

AHI	Apnea-Hypopnea Index
ACE	Angiotensin-converting enzyme
ADRB2	Adrenoceptor beta 2,
ApoE	Apolipoprotein E
BDNF	Brain-derived neurotrophic factor
BHS	Busselton Health Study
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CHF	Chronic heart failure
CI	Confidence interval
cM	Centimorgan
CPAP	Continuous positive airway pressure
CRP	C-reactive protein
CYBA	Cytochrome b-245, alpha polypeptide
EA	Effect allele
EDN	Endothelin genes
EDNRA	Endothelin receptor type A
EEG	Electroencephalogram
EKG	Electrocardiogram
EMG	Electromyogram
EOG	Electroculogram
FTO	Fat mass and obesity related gene
GDNF	Glial cell-derived neurotropic factor
GWAS	Genome wide association study
HP	Haptoglobin
IL-6	Interleukin 6
ISAC	Icelandic OSA cohort
LEPR	Leptin receptor
LOD	Logarithm (base 10) of odds
LPAR1	Lysophosphatidic acid receptor 1
MC	Meiotic clustering
MI	Myocardial infarction
MTCH2	Mitochondrial carrier 2
NEU3	Neuraminidase 3
OA	Other allele
ODI	Oxygen-Desaturation Index
OR	Odd ratio

OSA	Obstructive sleep apnea
PTGER3	Prostaglandin E3 receptor
PVRL2	Poliovirus receptor-related 2
REM	Rapid eye movement
RESP	Respiration
RR	Relative risk
SD	Standard deviation
SERT	Serotonin transporter
SLC6A4	Solute carrier family 6
SM	Severe, moderate
SMM	Severe, moderate, mild
SNA	Sympathetic nerve activity
SNP	Single nucleotide polymorphism
tBID-BAX	truncated BH3-interacting domain death agonist-apoptosis regulator BAX
TNF-alpha	Tumor necrosis factor-alpha
UCP2	Mitochondrial uncoupling protein 2
UCP3	Mitochondrial uncoupling protein 3
UPPP	Uvulopalatopharyngoplasty
USA	United States of America
WASHS	Western Australian Sleep Health Study

1 Introduction

Obstructive sleep apnea (OSA) is a common condition characterized by repeated episodes of pauses in breathing during sleep. These pauses are due to obstruction in the upper airway caused by complete or partial collapse of the airway (Figure 1) and are followed by loud snoring. Together with daytime sleepiness, loud snoring is the most common OSA symptom (1-3).

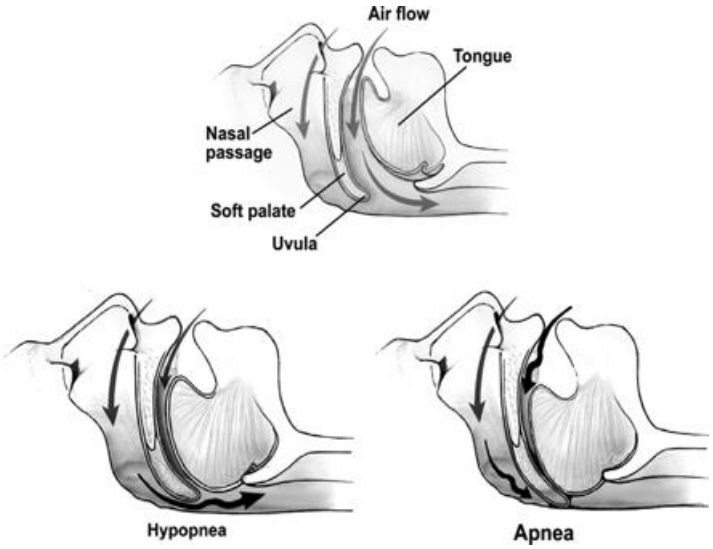


Figure 1. Anatomical view of the airway.

Normal airway (top), partial obstruction (bottom left) and complete obstruction (bottom right) of the airway. For the normal airway the air flows easily to the lung and the individual breathes normally. When the upper airway is partly or completely obstructive the airflow is disturbed and leads to snoring and disturbed sleep. The figure is from Somers et al. (4) with permission from Rightlinks

1.1 What is OSA?

Normal sleep is divided into two types; REM (Rapid eye movement) and non-REM sleep. During non-REM sleep the heart rate, blood pressure and sympathetic nerve activity decreases, whereas REM sleep is characterized by increased activity in the brain and sympathetic nerves (5). In OSA patients the homeostatic control during REM sleep is altered. Arousals in sleep caused by obstruction in the airway lead to activation of the sympathetic nerve system. Blood pressure and sympathetic nerve activity are high during sleep in OSA patients, and even when awake the sympathetic nerve system is more discharged in OSA patients than in healthy individuals (6). At the end of each OSA episode blood pressure elevates, heart rate and muscle tone increase and there is sympathetic nerve activation (Figure 2).

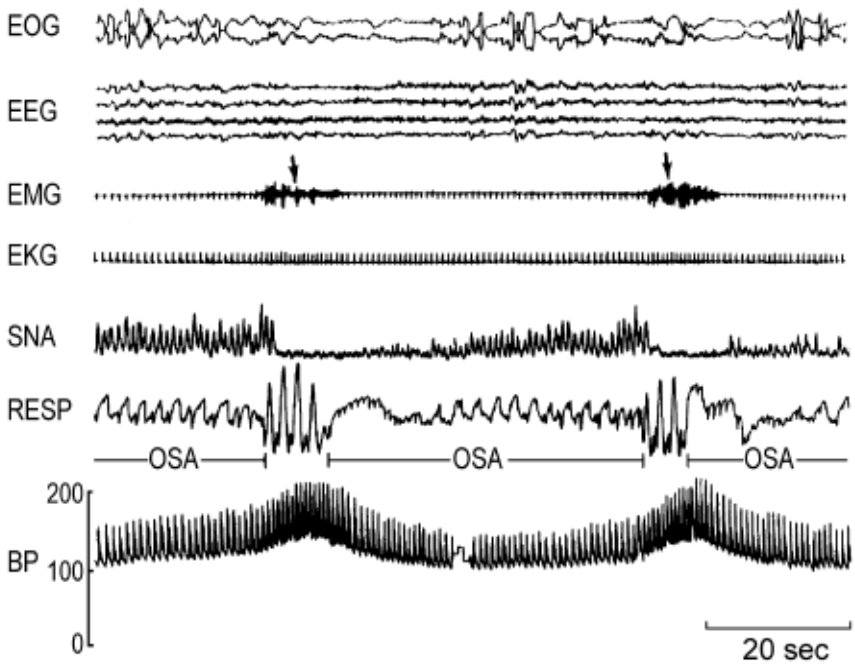


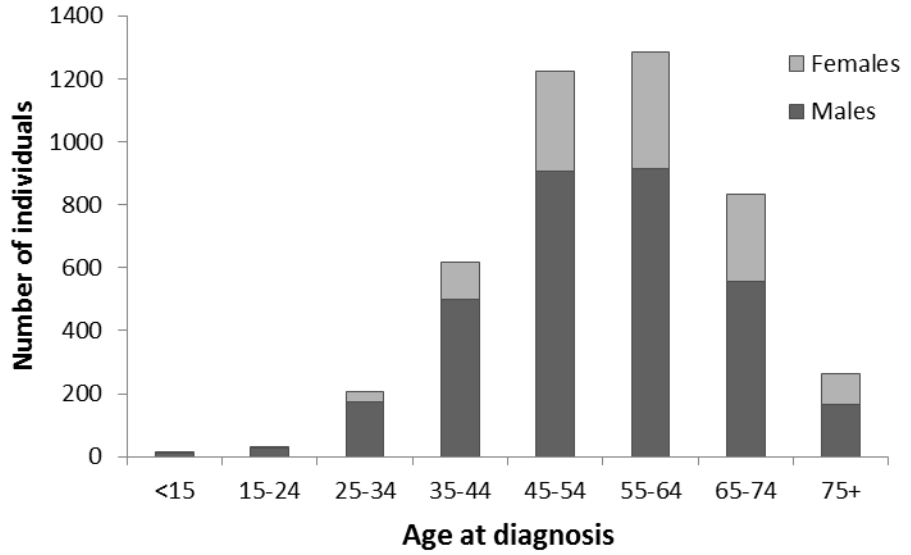
Figure 2. OSA sleep.

Recordings of the electroculogram (EOG), electroencephalogram (EEG), electromyogram (EMG), electrocardiogram (EKG), sympathetic nerve activity (SNA), respiration (RESP) and blood pressure (BP) during REM sleep in an OSA patient. SNA increases during the apnea but at the end of the apnea BP rises and muscle tone increases. EOG shows eye movements that slow down at the end of the apnea indicating arousal from REM sleep. The figure is from Somers et al. (6) with permission from Rightlinks

OSA patients often wake up several times during the night and many of them suffer from severe sleepiness during the daytime. They are more likely to doze or sleep during regular daily situations such as watching TV or reading and are more likely to have car accidents (1, 7). They are often irritated or even depressed.

Diagnosis of OSA is based on sleep studies, called polysomnography, where the individual is monitored and vital signs are recorded (8). From the polysomnography the existence and severity of the OSA is estimated as the number of apneas and hypopneas per hour of sleep (Apnea-Hypopnea Index (AHI)). Oxygen-Desaturation Index (ODI) is calculated in a similar manner as the number of oxygen desaturation (>4%) events per hour.

a)



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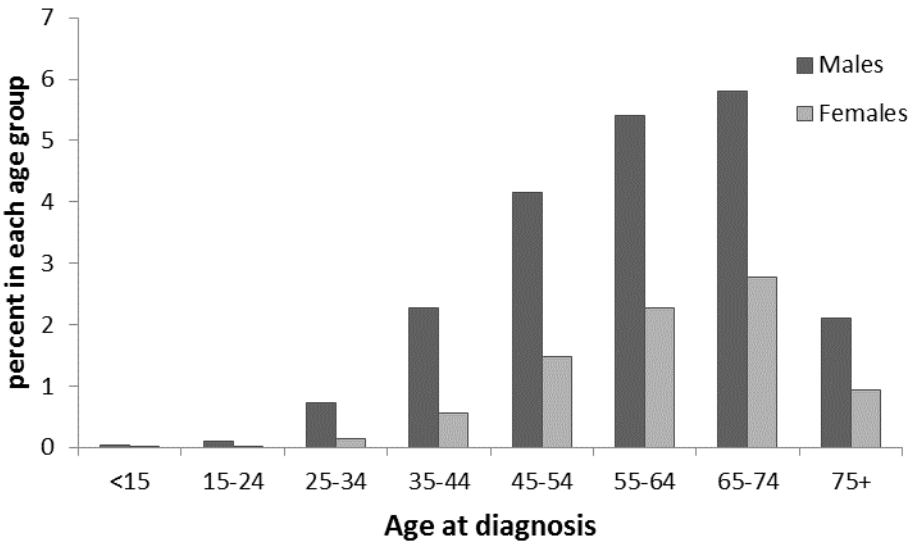

Figure 3. Distribution of age and gender in OSA in Iceland 2010.

a) Number of men and women diagnosed with OSA (n=4,477) at 10 year age intervals
b) Percentage of men and women in the population diagnosed with OSA at 10 year age intervals

OSA is more common in the middle-aged population than in younger people and 2-3 times more common in men than women (1). It is estimated to affect around 4% of middle-aged men and 2% of middle-aged women. A total of 4,477 individuals (3,253 males and 1,224 females) had been diagnosed with OSA in Iceland 2010, corresponding to 2.6% of those 35-65 years of age in the population, of whom 3.8% were men and 1.4% women (Figure 3). Most of the patients were diagnosed when middle-aged and at 55-74 years of age approximately 5-6% of men 2-3% of women were diagnosed with OSA.

Currently it is estimated that 6.2% of middle-aged men and 2.6% of middle-aged women in Iceland have OSA (Gislason T personal communication, 2012).

OSA patients have increased prevalence of other diseases, such as hypertension, myocardial infarction (MI), chronic heart failure (CHF) and stroke (1) compared to age and gender matched controls even when adjusted for obesity. In the Icelandic OSA cohort (ISAC), 59% of OSA cases have hypertension, 36% have MI or CHF, 11% have stroke and 66% have a body mass index (BMI, kg/m^2) of 30 or higher (Gislason T personal communication, 2012).

Several treatments are available for OSA. The most common is continuous positive airway pressure (CPAP) which is cheap and safe. The patient sleeps with a mask that delivers a stream of compressed air through the upper airway that with increased pressure prevents the airway from collapsing during sleep (Figure 4).

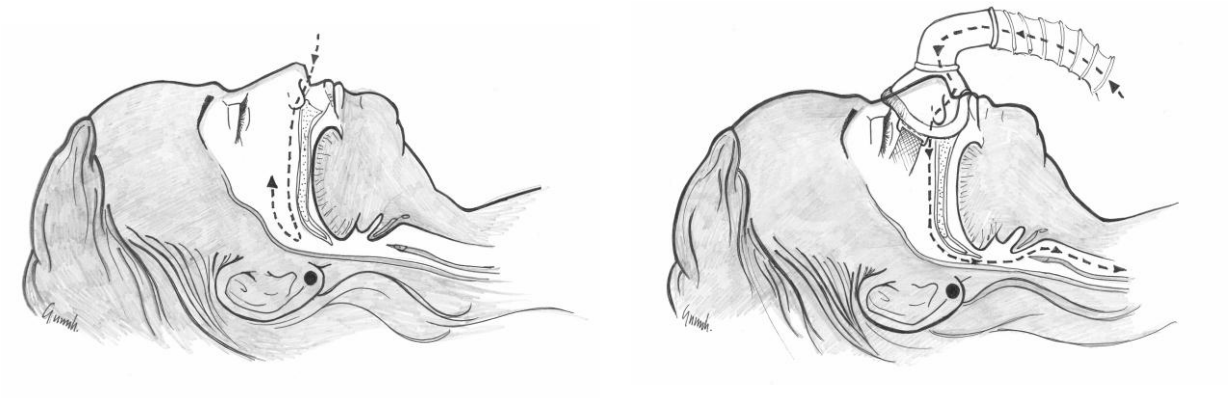

Figure 4. Treatment with CPAP keeps the airway open by delivering a stream of compressed air through the airway.

Untreated individual with OSA, the airway is blocked because of obstruction in the airway (left). CPAP-treated individual, the pressure from the CPAP machine keeps the airway open (right). Figures drawn by and used by the courtesy of Gunnhildur Þórunn Jónsdóttir

Most OSA patients benefit from using CPAP and many comorbidities become milder or disappear (4). CPAP treatment decreases daytime sleepiness (3, 9) and blood pressure and symphatic nerve activity during sleep (10-12). A comparative study showed that with therapeutic CPAP the overall mean blood pressure decreases where it increases with subtherapeutic CPAP, and that CPAP has more effect on severe OSA patients than on those with mild OSA (10).

If a patient fails to use or benefit from CPAP other treatments are available. There are many reasons for having an obstructive airway, such as a broken or skewed nose, enlarged tonsils or abnormal craniofacial structure. The most common surgery for OSA patients to alleviate their condition is partial removal of the uvula and redundant soft tissue of the soft palate (UPPP); however such surgeries have limited effects. Snoring may stop but breathing may continue to be disordered and therefore the UPPP

treatment may lead to silent apnea (13). Oral appliances can also be used to treat mild and moderate OSA, by adjusting the mandibular or the tongue with a mold that pushes the jawbone forward and keeps the airway open (4, 14). Analysis has shown that snoring decreases but the OSA only gets better in roughly half of the patients (4).

Weight loss is associated with improvements in OSA (15-17) but does not necessarily cure the condition so that other treatments may still be needed (16, 18). Studies of lifestyle intervention such as strict diet and exercise, have shown promising results in treating OSA (15, 17), as have studies on surgically induced weight loss (16, 18, 19). However, studies based on lifestyle intervention have had limitations, such as small sample sizes and unequal gender numbers, a too short follow-up time, lack of control groups and insufficient weight loss (16-18, 20), but weight loss should be considered as treatment for OSA patients who are obese.

1.2 Risk factors and comorbidities

1.2.1 Obesity

Due to increased energy intake and decreased physical activity, obesity is considered to be one of the biggest health problems we are facing worldwide. Nearly 43 million children under 5 years of age were overweight (BMI ≥ 25) in 2010 and over 1.5 billion adults were overweight in 2008, of whom 500 million were obese (BMI ≥ 30). A total of 700 million are expected to be obese by 2015 (21).

Obesity contributes to health problems and diseases, such as cardiovascular diseases and diabetes, and is a well-known risk factor for OSA. It is believed to alter breathing during sleep by multiple mechanisms (22). In addition to obstruction of the upper airway, excess weight reduces the lung volume, leading to more effort in breathing and uneven CO_2/O_2 exchange (23).

Fat distribution is considered an important factor for development of OSA (24, 25). Visceral adiposity better predicts OSA than overall obesity (22) and increased fat in the neck area can affect the upper airway. The adipose tissue around the upper airway compresses the airspace and contributes to airflow resistance that leads to apnea and hypopnea (26). Obese individuals with OSA tend to have more fat deposition in the tongue and soft palates than weight-matched controls (27) and even non-obese OSA (BMI<30) patients have increased fat in the parapharyngeal fat pads (28). Fat distribution explains some of the difference in OSA prevalence among men and women (1). Women have less adipose tissue around neck and abdomen than men, but with age fat increases around the neck for both genders (26). Menopause is associated with fat redistribution where abdominal fat is increased (29) resulting in higher risk of OSA among women in their menopause than before, but with hormonal replacement therapy the prevalence of OSA is decreased (30, 31).

1.2.2 Upper airway structure

The pharyngeal walls do not have bone support and variations in upper airway structure can cause obstruction of the airway. Patients with OSA may have abnormalities such as a larger tongue and soft palate and smaller, retruded jaw (Figure 5).

Craniofacial abnormalities have been linked to OSA (32-36), with the strongest link with non-obese OSA patients (33). Size and/or the position of maxilla, mandible and hyoid bone as well as tongue size and enlarged soft tissues have all been linked to OSA (27, 33, 37-39). Upper airway measurements explain 26%-33% of the variance in AHI, but for non-obese and younger OSA patients these measurements explain a full 55%-69% of the AHI variance (32).

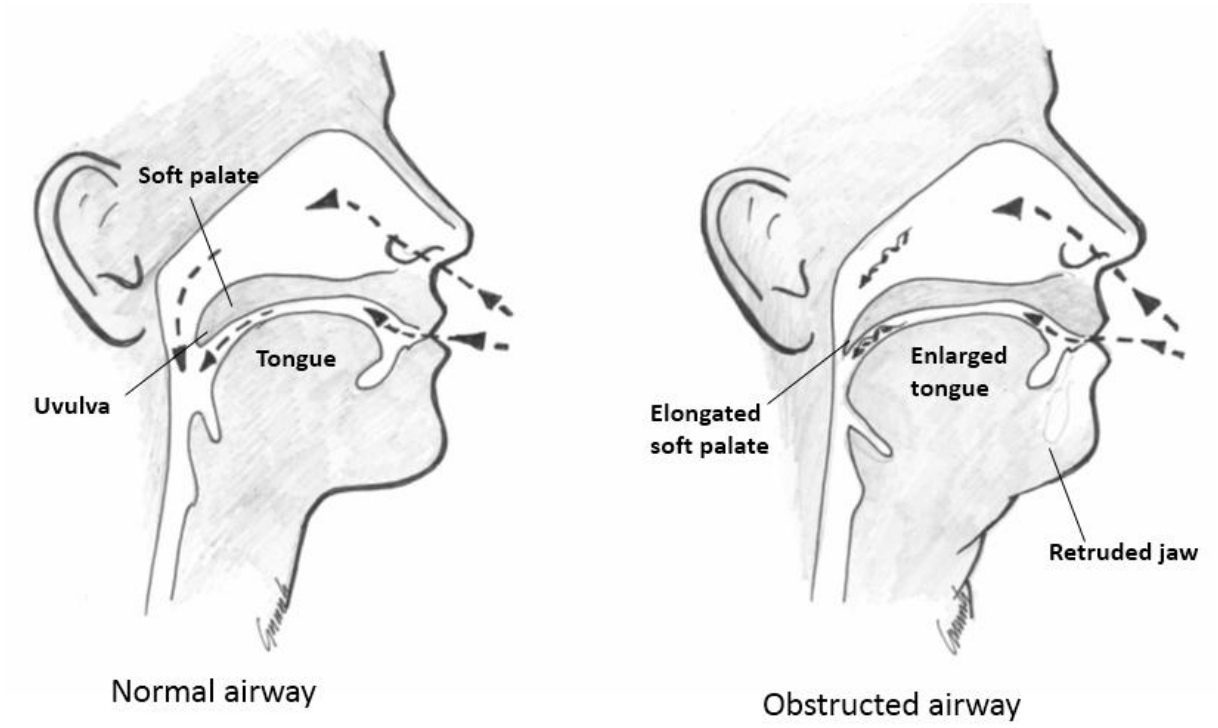

Figure 5. Normal airway and obstructed airway.

In a normal airway, uvulva, tongue and soft palate are of normal size with the tongue pointing forward (left). For individuals with OSA the soft palate is longer and the abnormalities of the jaw crowd the enlarged tongue (right). Figures drawn and used by the courtesy of Gunnhildur Þórunn Jónsdóttir

1.2.3 Cardiovascular diseases

The most common cardiovascular disease in OSA patients is hypertension that affects about 50% OSA patients (4). The relationship between hypertension and OSA is complex. Intermittent hypoxia, renin-angiotensin, chemoreceptor stimulation and activation of the sympathetic nervous system seem to be associated with hypertension and OSA where activation of the sympathetic system is thought to have

the biggest impact (40). During apnea blood pressure decreases, whereas following apnea blood pressure and heart rate increase significantly (41). The risk of hypertension increases by approximately 1% with every apneic event per hour of sleep (42). It has been suggested that OSA is common in patients with hypertension that is difficult to control with medication (43) and should be considered an independent predictor of drug-resistant hypertension in patients younger than 50 years (44).

OSA is also associated with coronary artery disease (CAD) and stroke, but how is not completely clear. OSA leads to arousal from sleep that causes increased sympathetic nervous activity. The upper airway obstruction also causes negative intrathoracic pressure and hypoxia, which in turn causes dysfunctions, more stress on the cardiovascular system and damage of the endothelial cells (4, 6).

With increased blood pressure, OSA also contributes to left ventricular failure where hypertension is an important risk factor. OSA can also contribute to cardiac heart failure with negative intrathoracic pressure and hypoxia. This is associated with increased thickness of the left ventricular wall and can in the long run lead to impaired left ventricular systolic function. Untreated OSA has a bad effect on the prognosis of patients with CAD. In a 5-year follow up study mortality was significantly higher in CAD patients who had OSA (41) and an 18-year follow-up study has shown that untreated OSA patients are five times more likely to die from cardiovascular diseases (7). There is some evidence that CPAP treatment can improve cardiac function (41).

1.3 Sleep studies in Iceland

In Iceland sleep studies have been performed since 1987, based on night studies, and the majority were performed in the subject's home using Embletta technology (EMBLA system, Flaga Medical Devices, Reykjavik, Iceland). Nasal airflow, chest and abdominal movements, pulse rate and oxygen saturation were measured as well as body position and activity. In recent years Embletta has been used for OSA diagnosis at five locations in Iceland but all patients who are diagnosed with OSA and need CPAP treatment are treated at the Department of Respiratory Medicine and Sleep at Landspitalinn, the National University Hospital of Iceland.

Assessment of clinical symptoms is based on a 5-point severity scale based on the Nordic Sleep Questionnaire (45). Information on the presence of comorbidities (hypertension, diabetes, etc.), and use of medication, has been collected systematically, as well as body weight and height measurements at the time of the sleep study. Extensive phenotypic information have been collected for this cohort and forms the basis for studies of sleep-related sweating in OSA patients (46), interaction of OSA and obesity on CRP and IL-6 (47), and insomnia in untreated OSA patients (48). More studies are ongoing.

1.4 Famililiality and genetic studies of OSA

1.4.1 Heritability and genetic studies

Heritability of diseases and traits can be demonstrated by familial aggregation (36, 49-54) and twin studies (55, 56). Identification of genes and sequence variants that confer risk to or protection from diseases has previously been done by twin studies (55), linkage analysis (57-61) and candidate gene approach (62, 63). Since 2006 genome-wide association (GWA) studies have led to identification of common sequence variants showing a small risk of diseases, such as atrial fibrillation (64), prostate cancer and type 2 diabetes (65) and obesity (66). The next generation sequencing has led to discovery of low frequency (1-5% minor allele frequency) and rare variants (<1% minor allele frequency) that confer a high risk of diseases like sick sinus syndrome (67), cancer (68, 69) and gout (70).

Some traits or diseases are known to be increased in some families although the genetic factors are not known. By analysing the familial aggregation it is possible to calculate the likelihood that a relative of a patient gets a disease or a trait, and to determine whether the risk is different from what can be expected by chance. The relative risk (RR) for close and distant relatives of an affected patient is calculated as the risk of relatives of the affected patient divided by the risk in the general population (49). DeCODE Genetic´s comprehensive genealogic database contains more than 75% of the one million Icelanders that have lived in Iceland since the Norse settlement in 874 AD. The database includes all living Icelanders and is complete for 10 generations. This makes it possible to investigate various degrees of relatedness among patients and create large control groups. The controls are chosen to match each person's birth year, gender, and number of ancestors going back five generations in order to avoid biases due to missing genealogical information. Each control set is then comparable with the patient list (49). Several familiality studies in Iceland have showed strong familial risk of diseases in Iceland, such as Parkinson's (49), various cancers (53, 71), and atrial fibrillation (50).

Previously, linkage analysis was a common approach to identify chromosomal regions harbouring causative genes (72). This was done by finding regions where affected relatives share genetic markers more often than would be expected by chance. The results are presented as nonparametric LOD scores (logarithm to the base 10 of the odds) where LOD scores greater than 3.6 are considered to show significant linkage and LOD scores of 2.0 - 3.0 are considered suggestive (73). A less stringent threshold increases the risk of false linkages. The markers that were used in the linkage studies at deCODE have an average density of 3-4 cM (74). They are all tested for robustness and ease of scoring and the analyses performed using complex algorithms (Allegro) (75) to calculate the probability of each inheritance vector providing the data. Since the average density of the markers is 3-4 cM the linkage analysis can only map a relatively broad region that may include many genes and therefore more detailed genotyping is needed to find the causative mutation by adding more markers to the region. At deCODE linkage studies on several diseases have been performed, such as for myocardial infarction and stroke (57, 59), hypertension (58) and osteoporosis (76).

Twin studies are based on monozygotic and dizygotic twins. Monozygotic twins have almost identical genome whereas dizygotic twins share about half of their genome, as is true of any other siblings. In addition to being born on the same day twins are most often raised in the same environment. These

features make twin studies helpful in understanding how environmental and genetic factors affect individuals and to estimate the heritability of common complex diseases (56). The heritability of a trait or disease or how much the trait or disease can be explained by genetic factors, can be estimated from the similarity between sets of monozygotic twins compared to the similarity between sets of dizygotic twins (55). The heritability of many traits and diseases such as height, weight, autism, obesity, asthma and alcohol use, have been shown in several twin studies (55, 56).

In contrast to the GWA studies where the whole genome is scanned, the candidate gene approach is based on selection of potentially interesting genes that are tested for association with the risk of a specific disease. This is usually a case-control study where variations in genes are compared between affected and healthy individuals. Unlike familiality and linkage studies, large families with affected and unaffected members are not needed. In fact, the cases and controls can be unrelated. However, such studies are often based on small cohorts and lack statistical power, and candidate gene studies have been criticized for lack of replication in independent sample sets (77). Apart from the small size of the cohorts tested, the lack of replication of the results can be due to variation in study design, different phenotype definition or false positive results. The genes that are considered as possible candidate genes are selected based on known physiological, biological or functional relevance to the disease and, therefore the lack of knowledge limits the conclusions that can be made.

Since 2006 GWA studies have dominated the research on human genetics of diseases and traits. GWA studies focus on association between diseases or traits and sequence variants in the genome. The case-control setup is the most common approach where the frequency of variants is compared between affected and healthy individuals. GWAs performed at deCODE have led to identification of common variants (>5% minor allele frequency) conferring a relatively small risk (odds ratio<2) to a large number of common complex diseases such as prostate cancer and type 2 diabetes (65, 78, 79), breast cancer (80), ovarian cancer (81), atrial fibrillation (64) and obesity (66). By combining several GWA studies in a meta-analysis, the sample sets are bigger and therefore the chances of identifying new variants are greater due to increased statistical power.

Next generation sequencing has dramatically increased the resolution of genome studies, where millions of previously unreported variants; SNPs (single nucleotide polymorphism), inserts and deletions and copy number variants, have been revealed. The cost of sequencing has decreased and data can be produced faster than ever. This has brought new opportunities to find rare risk variants that confer high risk for diseases such as the sick sinus syndrome (67), gout (70), various cancer (68, 69) and Alzheimer's (82) or provide information about human evolution (83).

From the whole genome sequencing, SNPs are called and genotyped with a two-step approach (67, 70). The SNPs that are identified are imputed to Icelanders that have been genotyped with chips with >300,000 SNPs by using long-range phasing information (84). In addition genotypes can partially be imputed into relatives of chip-genotyped individuals, using the fully phased genotypes available. Since the genotypes come from different chips the overlap of variants between them is not complete. The imputation is therefore carried out in two steps where the initial imputation step is carried out on each chip series separately to create a single harmonized, long-range phased genotype dataset. Subsequently, this genotype dataset is used in the second step of imputing the full set of 38.5 million

variants. After phasing and imputation, association analysis is performed with logistic regression, matching controls to cases based on the information score of the imputed genotypes (67-70).

1.4.2 Familiality of OSA

Several studies have shown that OSA aggregates in families (35, 36, 51, 52, 85, 86) and the relative risk is 1.5-2.0 for first degree relatives of OSA patients. In an Icelandic study from 2002 the relative risk for a first degree relative was 2.0 for all OSA and 2.3 for more severe OSA (CPAP treated) (52). Even after adjusting for BMI (36) or analysing non-obese individuals only (51) the risk is higher in relatives of OSA patients than in relatives of controls.

Children who have parents with OSA are more likely to be hospitalized due to sleep disorders than children whose parents do not have OSA (86). The sleep disorders can be due to either pediatric OSA or tonsillar hypotrophy, which has a lower but significantly increased risk. The risk is higher for boys than girls (86).

Craniofacial differences between OSA patients and controls have been demonstrated in several studies (27, 33, 35, 37, 54). First-degree relatives of non-obese OSA patients have abnormal craniofacial structure and smaller upper airways compared to the general population (34, 36). Total soft-tissue volume, tongue size and the size of the lateral pharyngeal wall aggregate in families (33) and are risk factors for sleep apnea (38).

1.4.3 Linkage studies of OSA

Linkage studies of OSA in the USA have not shown significant linkage with any chromosomal regions, but a few suggestive linkage regions have reported (60, 61, 87).

In the European-American families in the Cleveland Family Study, linkage to AHI was studied and 12 linkage peaks with a LOD score of >1 were observed with the highest LOD score of 1.64 (87). When adjusted for BMI, only two linkage peak had a LOD score of >1, one on chromosome 2p (LOD score 1.64 before adjusting for BMI, LOD score 1.33 after adjusting for BMI), and the other on chromosome 19p (LOD score 1.40 before adjusting for BMI, LOD score 1.44 after adjusting for BMI) (87). The chromosome 19p linkage region harbors the *ApoE* gene, which is considered a candidate gene to OSA; however finemapping of the region did not suggest ApoE to be a causative gene (62). A second study, based on the same families from the Cleveland Family Study and additional individuals, revealed only two linkage peaks on chromosome 6q and 10q (61). The LOD score decreased from 4.7 to 0.4 for the chromosome 6q locus, and from 2.7 to 0.7 for the chromosome 10q locus, when adjusted for BMI. However, the LOD score for one linkage peak at chromosome 6p11-q11 increased from 0.6 to 3.5 when adjusted for BMI, indicating an effect through a pathway independent of BMI (61).

In the studies cited, linkage to BMI was also studied and several linkage peaks with a LOD score of >1 were observed (60, 61). In the first study 15 linkage peaks with a LOD score of >1 were observed for BMI, but were reduced to four after adjusting for AHI, with the highest LOD score 1.64 (60). In the second

study, only one linkage peak was observed for BMI, at chromosome 17, which increased from a LOD score of 1.9 to 2.7 when adjusted for AHI (61).

Linkage studies of OSA among African Americans from the Cleveland Family Study (61, 87) showed only one linkage peak for AHI with a LOD score of >1 on chromosome 8q that decreased from a LOD score of 1.29 to 1.09 when adjusted for BMI (87). In a second study based on the same African Americans with additional individuals, the linkage peak on chromosome 8q did not appear, but three regions with a LOD score of >2 after adjusting for BMI were observed, on chromosomes 8p, 18q and 22q (61).

All these studies were small, based on 349 (60) and 641 (61) European-Americans with OSA and 277 (87) and 634 (61) African-Americans with OSA. In such small studies significant results are not expected due to lack of statistical power. Since they are based on overlapping family cohorts the latter studies in both European-Americans and African-Americans are not an independent replications. Several plausible candidate genes in the regions under the linkage peaks were mentioned but no finemapping was done (60, 61, 87) apart from the *ApoE* gene which was not found to be causative for OSA (62).

1.4.4 Obesity-related genes

It has been argued that due to the strong effect of obesity on obstructive sleep apnea it will be difficult to identify genetic factors contributing to the risk of OSA through other mechanism and pathways. However, by adjusting for BMI it should be possible to find sequence variants in genes of obesity-independent pathways that confer risk to OSA if the sample size is large enough. Many obesity-linked loci have been identified through candidate gene studies (88, 89) and with GWA studies (66, 90-93). Meta-analyses have led to identification of additional obesity-related loci (94, 95). However, the effects of these variants on obesity are small and do not fully explain the condition, indicating that obesity is a complex trait with genetic and environmental causes.

The best known obesity-related gene is *FTO* (fat mass and obesity related gene) (88) which has been replicated in several studies. The *FTO* gene is widely expressed in the brain (66,88,90) and highly expressed in the hypothalamus (90). Alteration of *FTO* expression in the hypothalamus influences food intake (96) and knockout of *FTO* shows that it controls energy expenditure (97).

Genome-wide association studies have identified additional loci that associate with obesity or obesity-related traits like BMI or weight. Altogether, 32 BMI loci show significant association with obesity or obesity-related traits, although only a small proportion of the BMI can be explained with these 32 variants (1.45%) where the FTO variant has the largest effect (0.34%) (94). Association of these obesity related sequence variants with OSA has not been reported so far (as of August 2013).

1.4.5 OSA candidate genes

Many obesity-linked genes have been suggested as associating with OSA, as well as genes involved in pathways affecting craniofacial morphology, bone and soft tissue, ventilator control, inflammation, sleep

and circadian rhythm (34, 63). Several studies of plausible OSA-linked genes have been done, both large case control studies, of many variants in many genes and smaller studies focusing on a few genes and few variants.

A case-control study on 52 candidate genes tested in 694 European-Americans for an association with AHI only showed significant association with three SNPs in two genes, *CRP* (encoding C-reactive protein) and *GDNF* (encoding glial cell-derived neurotropic factor) (63). When tested in the Icelandic OSA cohort (1,711 OSA cases with AHI ≥ 5 and 24,981 controls) these SNPs did not associate with OSA, nor did variants in the other 50 candidate genes (98). In another study of SNPs in approximately 2,100 candidate genes relevant to heart, lung, blood and sleep disorders, only one SNP in the *PTGER3* gene (encoding prostaglandin E3 receptor) showed significant association with OSA (AHI≥15) in roughly 950 European Americans. This SNP showed a stronger association with OSA after adjusting for BMI, but was not replicated in another OSA cohort (99). In African Americans another SNP in the *LPAR1* gene (encoding lysophosphatidic acid receptor 1), showed significant association with OSA and nominal association in the replication cohorts (99).

Leptin, inflammatory markers and sleep-related hormones have been linked with OSA. Leptin plays an important role in body weight and food intake, and has been suggested as affecting respiratory control or the upper airway (100). An increased leptin level is associated with a higher BMI (101), but studies of leptin in OSA patients have shown contradictory results (102, 103). No sequence variants in the genes encoding leptin and a leptin receptor have shown significant association with OSA (63).

Increased levels of inflammatory molecules like *CRP*, *IL-6* (encoding interleukin 6) and *TNF-α* (encoding tumor necrosis factor-alpha) have been linked to OSA (47, 63, 104-106) as well as several genes that are believed to be involved in ventilator control like EDN genes (encoding endothelium). The endothelium is important for the cardiovascular system and *EDN1* has been mentioned as an interesting target, as it is a potent vasoconstrictor and pro-inflammatory peptide (107). However, *EDN1*, *EDN3* and *EDNRA* (encoding endothelin receptor A) have not shown significant association with OSA (63).

Sleep is regulated by the hormone melatonin. Its original form, tryptophan, is converted in the brain to serotonin and then to melatonin. Sequence variants in several serotonin receptor and transporter genes have not shown association with OSA in European- and African-Americans (63) or in the large Icelandic OSA cohort (98).

1.4.6 GWAS of OSA

So far no genome wide association studies on OSA have been reported. At deCODE over 5,400 OSA patients have been typed on the Illumina Human Hap300, Human Hap300-duo (317K), Human CNV370-Duo (370K), Human Omni Express or Human Omni 2.5 Bead arrays (Illumina Inc., San Diego, CA). GWAS on OSA based on these data is beyond the scope of this study.

2 Aims

The overall aim of the study was to identify novel sequence variants that associate with obstructive sleep apnea (OSA) in order to identify genes and pathways that contribute to the risk of OSA in order to enhance our understanding of the pathogenesis of OSA and help to develop novel diagnostic and therapeutic measures.

There were four specific aims:

I. To assess the familiality of OSA in Iceland and estimate the relative risk of OSA for relatives of OSA patients, for the main OSA phenotypes and for BMI–related subphenotypes.

II. To identify chromosomal regions or loci linked to OSA using linkage analysis and map the causative genes by finemapping significant and suggestive linkage regions using case control analysis of single nucleotide polymorphishms (SNPs)—from Bead-chip array genotyping.

III. To analyse the effects of obesity-related sequence variants on the risk of OSA, since obesity is closely linked with OSA.

IV. To validate sequence variants in genes reported to associate with OSA in the large Icelandic OSA sample set.

3 Methods

3.1 Patients

3.1.1 OSA patients and sleep studies in Iceland

A total of 4477 Icelanders (1224 females and 3253 males) with a confirmed diagnosis of OSA from 1987 to 2010 were included in the familiality study along with their relatives. The patients had been diagnosed in a sleep study and the majority were or had been treated with CPAP. Of these 4,477 individuals, 3,541 patients (969 females and 2572 males) had signed informed consent forms and were included in the linkage and obesity-related candidate gene studies. The OSA groups analysed are shown in table 1 and the characteristics of the patients are shown in table 2.

Of the 7500 patients diagnosed with OSA by August 2013, over 5,400 patients have been typed on the Illumina Human Hap300, Human Hap300-duo (317K), Human CNV370-Duo (370K), Human Omni Express or Human Omni 2.5 Bead arrays (Illumina Inc., San Diego, CA) (August 2013). Phasing and imputation of these genotypes were included in the obesity-related SNP study and OSA candidate gene study (Table 1).

The study was approved by the Data Protection Authority and the National Bioethics Committee in Iceland. Personal identifiers associated with medical information and blood samples were encrypted with a third-party encryption system (Identity Protection System, IPS) as previously described (108).

Table 1. The Icelandic OSA cohort, number of individuals with phenotypes tested in familiality, linkage analysis and finemapping

Phenotypes		Familiality	Linkage /Obesity	Finemapping	Candidate genes/Obesity
		#PN	#PN	#PN	#PN
	All OSA	4,477	3,102	n/a	7,508
	SMM	3,670	2,843	2,148	4,054
	SM	3,125	2,419	n/a	3,422
BMI≥30	**All OSA**	2,353	1,790	n/a	3,122
	SMM	2,189	1,696	1,307	2,415
	SM	1,922	1,480	1,168	2,107
BMI<30	**All OSA**	1,554	1,220	1,127	2,455
	SMM	1,407	1,123	836	1,557
	SM	1,143	919	n/a	1,251

Number of individuals with phenotypes tested in familiality, linkage analysis, obesity-related genes, finemapping of linkage regions and the candidate gene study. ALL OSA: AHI and/or ODI ≥ 5 and/or CPAP users, SMM (severe, moderate, mild): AHI and/or ODI ≥ 5, SM (severe, moderate): AHI and/or ODI ≥ 15, n/a = not used in the analyses

Table 2. Characteristics of Icelandic, Australian and USA OSA patients used in the linkage, finemapping and obesity-related SNP analysis.

Study:	**Linkage**		**Linkage/Obesity**		**Obesity**		**Linkage finemapping**					
Phenotype:	**ALL OSA***		**SMM****		**SMM**		**Obese SMM OSA subjects**					
Cohort:	**Discovery Iceland**		**Discovery Iceland**		**Replication Australia**		**Discovery Iceland**		**Replication Iceland**		**Replication USA**	
	Male (N=3,253)	**Female** (N=1224)	**Male** (N=2,263)	**Female** (N=862)	**Male** (N=1,008)	**Female** (N=619)	**Male** (N=903)	**Female** (N=404)	**Male** (N=425)	**Female** (N=119)	**Male** (N=177)	**Female** (N=124)
Characteristic	Mean ± SD											
Age, years	54.8 ± 12.1	58.5 ± 11.7	55.3 ± 11.8	60.0 ± 11.3	54.0 ± 14.5	54.5 ± 13.7	55.6 ± 11.4	58.4 ± 10.9	51.7 ± 11.5	56.7 ± 11.2	52.7 ± 11.9	53.2 ± 11.1
BMI, kg/m²	32.0 ± 5.9	33.3 ± 7.8	32.3 ± 5.9	33.1 ± 7.7	31.1 ± 6.2	32.8 ± 8.8	35.5 ± 4.6	32.0 ± 20.3	35.6 ± 4.7	37.3 ± 6.2	37.0 ± 7.0	40.2 ± 8.5
AHI, events/hr	37.0 ± 22.3	30.1 ± 21.2	37.4 ± 21.8	29.9 ± 20.0	35.1 ± 28.9	25.8± 23.1	43.4 ± 23.4	28.2 ± 21.3	41.8 ± 22.8	33.2 ± 24.1	48.7 ± 32.6	31.2 ± 27.0
ODI (4%) events/hr	29.1 ± 21.2	25.1 ± 21.0	29.7 ± 21.4	25.5 ± 20.8	N/A	N/A	35.3 ± 22.4	37.2 ± 6.3	36.4 ± 23.2	32.3 ± 24.1	36.5 ± 28.6	20.2 ± 20.9

Characteristics of the OSA patients in the main phenotypes tested, mean and standard deviation (SD) of age, BMI, AHI and ODI for the genders separately.

ALL OSA*: AHI and/or ODI ≥ 5 and/or CPAP users, SMM** (severe, moderate, mild): AHI and/or ODI ≥ 5. BMI: Body mass index, AHI: Apnea-hypopnea index, ODI: Oxygen-desaturation index, NA=Not available

3.1.2 Replication sample sets

3.1.2.1 OSA patients and controls from Pennsylvania, USA

A total of 304 European-American obese (BMI ≥ 30) OSA patients (AHI ≥ 5; 177 males and 124 females) and 392 controls were used to validate the findings in the Icelandic population.

The characteristics of the patients are shown in table 2. The patients had undergone sleep studies and signed an informed consent. Controls were selected from participants of the cardiac catheterization study program at the University of Pennsylvania Medical Center in Philadelphia (PENN CATH). The control group represented individuals who did not have significant luminal stenosis on coronary angiography (luminal stenosis >50%) or a history of myocardial infarction (109).

The Institutional Review Board of the University of Pennsylvania approved this study.

3.1.2.2 OSA patients and controls from Western Australia

The Western Australian Sleep Health Study (WASHS) and Busselton study (BHS) were used to validate the findings in the Icelandic population.

A total of 1,627 OSA patients (1,008 males and 619 females) and 4,893 controls were used in this study. WASHS is a collaborative project focused on the genetics of sleep disorders, in particular sleep apnea, based on questionnaire data, sleep-associated data and blood samples (110). The cohort was predominantly European Australian and comprised of patients with OSA (91%). The WASHS cohort was population based, where the numbers of mild, moderate and severe patients were almost equal. The characteristics of the patients are shown in table 2. Controls were from the BHS that have been involved in a series of health surveys since 1966 (111).

3.2 Genotyping

3.2.1 Microsatellite genotyping

The PCR amplifications were prepared, run, and pooled using Zymark SciClone ALH 500 robots with a similar protocol for each marker. The reaction volume was 5 µl, and, for each PCR, 20 ng of genomic DNA was amplified in the presence of 2 pmol of each primer, 0.25 U AmpliTaq Gold, 0.2 mM dNTPs, and 2.5 mM MgCl2 (buffer was supplied by the manufacturer). Cycling conditions were 95°C for 10 min, followed by 37 cycles at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. The PCR products were supplemented with the internal size standard, and the pools were separated and detected on an Applied Biosystems model 3730 sequencer using Genescan (v. 3.0) peak-calling software. Alleles were automatically called with an internal allele-calling program (74).

3.2.2 SNP genotyping

The Icelandic OSA patients and controls were genotyped using the Illumina Human Hap300-duo (317K) or Human CNV370-Duo (370K) Bead arrays (Illumina Inc., San Diego, CA). The SNPs on the Illumina chips used in the analyses, passed quality filters (yield >95%, minor allele frequency >0.01, no distortion of Hardy–Weinberg equilibrium (HWE) in controls (P>1.0×10^{-3}). All samples had call rates above 98%.

Single track SNP genotyping for the Icelandic and USA samples was carried out by deCODE genetics by applying the Centaurus (Nanogen) platform (112). The quality of Centaurus SNP assays was evaluated by genotyping each assay in the CEU HapMap samples and comparing the results with the HapMap data. Assays with a >1.5% mismatch rate were not used.

The Australian OSA patients and controls were genotyped at Molecular Genetics, PathWest Laboratory Medicine of West Australia using the Centaurus platform. The quality of Centaurus SNP assays was evaluated by sequencing three samples, one from each genotype, for each SNP and comparing the results with the Centaurus data.

3.3 Familiality calculation

Statistical algorithms were used to find in the Icelandic genealogy database all ancestors related to each OSA patient within a given number of generations and to identify all relatives of a given relation, such as siblings, aunts or uncles (49). To evaluate familiality the relative risk for close and distant relatives was calculated using statistical methods previously described (71).

The relative risk for the first- to fifth-degree relatives is an estimation of occurrence of disease in relatives compared with incidence in the general populations. To assess the significance of the relative risk obtained for a given degree or type of relative, their observed values were compared with the relative risk computed for 1,000 independently drawn and matched groups of control individuals. The controls were drawn randomly from the genealogical database with the same gender, year of birth and number of ancestors recorded in the database as the patient to whom they were matched. A reported P = 0.05 for the RR would indicate that 50 of the 1000 matched control groups had values as large or larger than that for the patient's relatives or spouses. When none of the values computed for the control groups was larger than the value for the patient's relatives or spouses, P<0.001 is reported.

3.4 Linkage

A genome wide scan was performed on OSA patients and relatives by genotyping the same 1,100 fluorescently labeled microsatellite marker set. All markers were tested for robustness and ease of scoring and with an average density of 3-4 cM, with genetic locations based on the deCODE map (74). The analyses were performed using Allegro (75), which uses complex algorithms to calculate the probability of each inheritance vector, given the data. The results shown are based on 4-6 meiotic clustering, with the condition that the numbers of families were ≥ 100, with ≥ 200 affected individuals.

3.5 Association analyses

Association between OSA and OSA subphenotypes and a genotype was tested by fitting a logistic regression using the allele count as an explanatory variable. The effects were adjusted for BMI and gender by including those covariates as explanatory variables in the logistic regression model. To adjust for possible population stratification and the relatedness amongst individuals we scaled the test statistics by the method of genomic control (113, 114). Adjusted P values were calculated by dividing the corresponding Chi2 values by the inflation factor. All P values are reported as two- sided.

4 Results

4.1 Familiality of OSA

Familial aggregation of 4477 individuals with OSA (AHI/ODI ≥ 5 and/or have been on CPAP treatment) were analysed for the whole group and for subgroups based on BMI.

OSA was shown to aggregate in families (Table 3). Relatives of OSA patients are more likely to have OSA than an individual in the general population and the risk is higher for the close relatives than for the more distant. Estimation of the relative risk (RR) for the relatives of OSA patients is shown in table 3. First-degree relatives of all OSA patients had more than a two-fold risk of having OSA when compared to the general population. The RR for the second and the third-degree relatives were significantly increased although lower than for the first-degree relatives (Table 3).

Table 3. Familial risk of OSA for relatives and spouses

	OSA all (N=4,477)		
relation	RR (95%CI)	P Value	No. of relatives
1st Degree	2.37 (2.13,2.49)	<0.001	36,303
2nd Degree	1.44 (1.29,1.51)	<0.001	123,070
3rd Degree	1.30 (1.19,1.31)	<0.001	308,785
4th Degree	1.17 (1.08,1.16)	<0.001	714,650
5th Degree	1.14 (1.06,1.11)	<0.001	1,657,116
Spouses	1.91 (1.51,2.28)	<0.001	5,089

The table shows numbers of relatives for first- to fifth-degree relatives and spouses, their risk ratio (RR) and 95% confidence intervals (in brackets) for all OSA patients (AHI and/or ODI ≥5 and/or CPAP users).

Obesity was shown to add to the risk of OSA. The first-degree relatives of obese OSA patients had more than a threefold risk of being obese with OSA compared to the general population, but the risk dropped to 1.5-fold in the second- and third-degree relatives (Table 4). First-degree relatives of non-obese OSA patients also had a significantly increased risk of having OSA, and the risk was greater than 1.0 for all relatives. For each degree of relation the RR was higher for relatives of obese OSA patients than non-obese (Table 4).

Spouses also showed increased risk of having OSA compared to the general population; however it should be noted that the group was smaller and with the confidence interval larger than for the other groups (Tables 3 and 4)

Table 4. Familial risk of OSA for relatives and spouses and effect of obesity

	OSA all with BMI≥30 (n=2,368)			OSA all with BMI<30 (n=1,576)		
Relation	RR (95%CI)	P Value	No. of relatives	RR (95%CI)	P Value	No. of relatives
1st Degree	3.21 (2.75,3.54)	<0.001	19,137	2.36 (1.85,2.84)	<0.001	13,212
2nd Degree	1.64 (1.38,1.84)	<0.001	65,030	1.45 (1.11,1.75)	0.002	44,955
3rd Degree	1.45 (1.27,1.51)	<0.001	163,882	1.15 (0.96,1.26)	0.071	110,797
4th Degree	1.24 (1.11,1.27)	<0.001	382,139	1.17 (1.02,1.24)	0.012	252,179
5th Degree	1.18 (1.08,1.18)	<0.001	880,573	1.17 (1.04,1.18)	0.001	590,288
Spouses	2.14 (1.41,3.04)	<0.001	2,673	1.40 (0.61,2.57)	0.177	1,857

The table shows number of relatives for first- to fifth-degree relatives and spouses, their risk ratio (RR) and 95% confidence intervals (in brackets) for obese (BMI≥30) and non-obese (BMI<30) OSA patients.

4.2 Linkage analysis

We did not observe genome wide significant LOD scores for any of the OSA phenotype tested. In total ten linkage peaks with LOD scores >1.5 were observed for the 4-6 meiotic clustering (MC) (Table 5).

For the main OSA group (All OSA) two linkage peaks, at 5p14 and 11q25, were observed. The LOD score was 2.21 for the locus at 5p14, but was not seen (LOD<1) when dividing the OSA patients into obese and non-obese subgroups. The linkage peak at 11q25 (LOD score 1.84 for all OSA) was also seen in obese OSA patients (LOD score 1.96). An additional five linkage peaks were observed for obese OSA patients (at 2q36, 7p21, 10q26, 11p14, 11q13). The linkage peak at 11p14 had the highest LOD score (LOD score 2.40) of the 10 linkage peaks identified. The second highest LOD score (LOD score 2.28) was at 11q13 (Figure 6). For non-obese OSA patients three linkage regions were identified (at 7p14, 12q24 and 19p13) with the highest LOD score of 2.1 at 19p13 (Table 5). LOD scores for the 10 linkage peaks identified are shown in table 5.

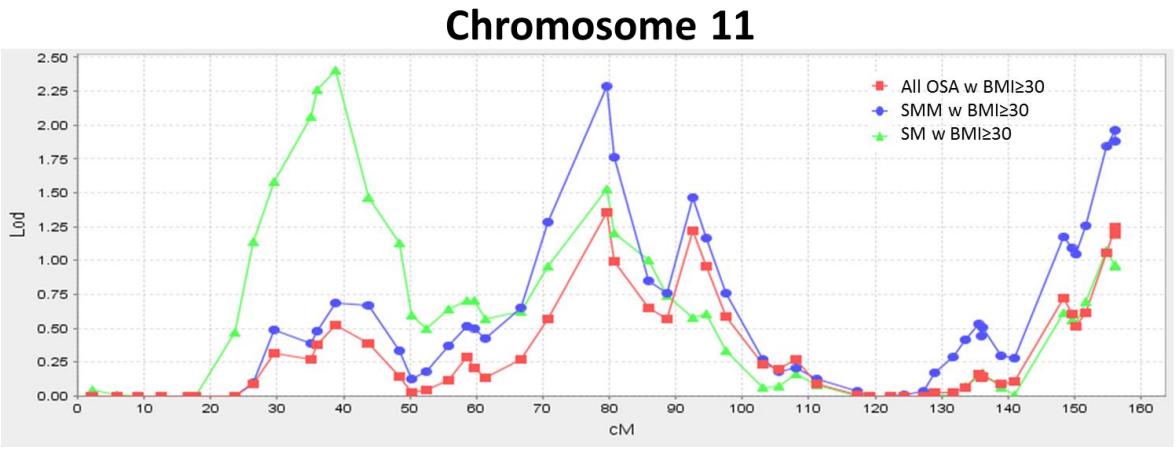

Figure 6. **Linkage plot of chromosome 11 for obese OSA patients.**

Three regions at 40 cM, 80, cM and 155 cM had LOD > 2 and can be considered suggestive.

Table 5. OSA phenotypes and linkage peaks with LOD scores ≥1.5.

		2q36	5p14	7p21	7p14	10q26	11p14	11q13	11q25	12q24	19p13
1 LOD Drop (kb)		221-236	16-30	15-21	31-45	132-134	19-26	63-78	130-133	128-131	12-19
1 LOD Drop (cM)		225-250	36-51	29-38	53-70	176-182	29-44	70-85	148-156	165-175	33-48
	ALL OSA		1.48#								
	SMM		**2.21†**						1.84*		
	SM		1.66#†						1.33*		
BMI≥30	**ALL OSA**	1.32*				1.2*		1.35*	1.25*		
	SMM	**1.88***		1.11*		**1.62***		**2.28***	**1.96***		
	SM			**1.63***		1.3*	**2.4**#	2.13*			
BMI<30	**ALL OSA**				1.27#					1.16#	**2.1***
	SMM				**1.74**#					**1.66†**	2.06*
	SM				1.24#					1.42#	

ALL OSA: SMM and/or CPAP users (AHI and/or ODI≥5 and/or CPAP users), SMM: AHI and/or ODI≥5, SM: AHI and/or ODI≥15. BMI: Body mass index (BMI≥30: obese). AHI: Apnea-hypopnea index, ODI: Oxygen-desaturation index. The highest LOD scores for each locus are showed in bold.
*:4MC, #:5MC, †:6MC

4.2.1 Finemapping

SNPs present on the Illumina Human Hap300-duo (317K) or Human CNV370-Duo (370K) Bead arrays under each of the 10 linkage peaks (1 LOD drop) identified were tested for association with the corresponding OSA phenotype (Table 6). A significant association of the variant rs681566[A] at 11p13

with obese SMM OSA ($P=1.26x10^{-6}$, OR=1.30) was found (Table 6). The rs681566[A] variant is in the first intron of the gene *NEU3* that encodes for a neuraminidase 3 that is ganglioside sialidase and has a critical regulatory function on the sialoglycosphingolipid pattern of the cell plasma membrane (115). The SNP was tested in additional OSA samples from Iceland and USA. Although the effects were in the same direction as in the discovery sample set, association with OSA in the replication sample sets was not significant (P=0.116, OR=1.14 in Iceland and P=0.384, OR=1.14 in USA) (Table 7). The association for discovery and replication samples combined was $P=6.98x10^{-7}$, OR=1.241 (1.139-1.351) (Table 7).

Table 6. Summary of regions and markers investigated in the linkage and fine-mapping analyses.

Chromosome Region	*Linkage Region with 1 LOD Drop (MB)*	*Number of SNPs for fine-mapping*	*Phenotype for fine-mapping*	*Number of cases for fine-mapping*	*lowest observed p-value*	*OR*	*SNP*
2q36	221,155,000 - 236,354,600	1,848	Obese SMM	1,307	1.23×10^{-3}	1.15	rs836235
5p14	16,247,400 - 30,388,300	1,190	SMM	2,148	2.57×10^{-4}	1.15	rs7732527
7p21	14,976,300 - 21,486,400	870	Obese SM	1,168	1.65×10^{-3}	1.21	rs9648196
7p14	31,496,500 - 45,655,900	1,569	Non obese SMM	836	5.21×10^{-4}	1.20	rs10225173
10q26	131,904,800 - 133,665,000	289	Obese SMM	1,307	2.81×10^{-3}	1.13	rs2944478
11p14	19,287,500 - 25,981,800	787	Obese SM	1,168	2.53×10^{-3}	1.18	rs1353649
11q13	63,373,900 - 78,490,300	1,254	Obese SMM	1,307	**1.26×10^{-6}**	1.30	rs681566
11q25	130,041,000 - 133,474,200	545	Obese SMM	1,307	1.62×10^{-4}	1.18	rs7108867
12q24	128,157,300 - 131,489,700	486	Non obese SMM	836	1.19×10^{-3}	1.23	rs418615
19p13	12,167,700 - 19,046,300	698	Non obese ALL OSA	847	9.54×10^{-4}	1.27	rs2287699

Shown for each chromosome region are the positions of the linkage regions (build 36) and the number of SNPs (from the Illumina 300K and 370K bead chips) used for finemapping each region. The phenotype that gave the highest LOD score for each region is shown and the number of cases that were used for finemapping. More than 32,000 controls were used in this analysis. The best SNP for each locus with p-value and odds ratio (OR) is reported. The thresholds for significant p-values were found with the Bonferroni correction based on number of SNPs tested. Significant p-value is shown in bold.

Table 7. Association of the variant rs681566[A] in the NEU3 gene at 11p13 with obese SMM OSA patients in the Icelandic and American sample sets.

Cohorts	*Cases/controls*	*P-value*	*OR*
Iceland	1,307/33,376	1.26×10^{-6}	1.30
Iceland-replication	544/5,473	0.116	1.14
USA	303/392	0.384	1.14
combined		6.98×10^{-7}	1.241 (1.139-1.351)

P-values and odds ratio (OR) for the Icelandic discovery cohort, the two replication cohorts from Iceland and the USA and combined for all cohorts.

4.3 Obesity-related variants in OSA

Thirty-two variants known to associate with obesity or obesity-related traits in previously reported genome association studies (66, 90, 94) were tested for assocation with OSA in Iceland and based on imputed data (Table 8).

Only rs1558902[A] in the *FTO* gene, was associated with SMM OSA (AHI and/or ODI ≥ 5; $P = 0.0009$, OR= 1.096) after Bonferroni correction for the number of variants tested. However, when adjusted for BMI and gender the association disappeared ($P = 0.76$) (Table 8).

After adjusting for BMI and gender the association of rs10838738[G] in the *MTCH2* gene encoding mitochondrial carrier 2, at 11p11 with OSA was strengthened ($P = 8.5 \times 10^{-5}$, OR = 0.879) (Table 8). It is notable that rs10838738[G] associated with reduced risk of OSA in contrast to what would be expected based on its effect on increasing BMI (90). None of the other BMI-related variants tested showed a significant association with OSA (Table 8). Age at diagnosis had no effect on the associations of any of the SNPs with OSA phenotypes (data not shown).

The *MTCH2* variant rs10838738[G] was further tested in an Australian sample set (see characteristics in Table 2) where the association with reduced risk of OSA was replicated when adjusted for gender ($P = 0.054$, OR = 0.920) and when adjusted for gender and BMI ($P = 0.044$, OR = 0.908). Association of rs10838738[G] with OSA in the Icelandic and Australian sample sets combined was stronger when adjusting for BMI ($P = 4.4 \times 10^{-6}$, OR = 0.884) than without BMI adjustment ($P = 0.00032$, OR = 0.913).

Severity of OSA often coincides with high BMI and therefore it was tested whether the effects of rs10838738[G] on the risk of OSA differed if the analysis was restricted to more severe OSA (SM defined as AHI and/or ODI ≥ 15). Furthermore, association of rs10838738[G] with various subgroups within the SM OSA group (males, females, obese, non-obese individuals) was tested and a reduced risk of OSA with similar ORs was found in all subgroups (Table 9). When the frequency of rs10828738[G] was compared between different sub-groups of OSA no significant differences were found (Table 10).

Table 8. Association of 32 obesity risk variants with obstructive sleep apnea.

						SMM		Adj. Sex		Adj. Sex+ BMI	
Chr	Position	SNP	Nearest gene(s)	EA/OA	Freq.	P	OR	P	OR	P	OR
1	72,585,028	rs2815752	NEGR1	A/G	0.58	0.27	1.03	0.33	1.03	0.96	1.00
1	74,764,232	rs1514175	TNNI3K	A/G	0.55	0.97	1.00	0.85	1.00	0.46	1.02
1	96,717,385	rs1555543	PTBP2	A/C	0.63	0.01	1.08	0.014	1.07	0.085	1.06
1	176,156,103	rs543874	SEC16B, RASAL2	G/A	0.2	0.69	1.01	0.71	1.01	0.82	0.99
2	612,827	rs2867125	TMEM18	C/T	0.84	0.26	1.04	0.3	1.04	0.73	0.99
2	25,011,512	rs713586	RBJ	C/T	0.46	0.82	0.99	0.65	0.99	0.21	0.96
2	59,156,381	rs887912	FANCL	C/T	0.74	0.87	1.01	0.92	1.00	0.61	1.02
2	142,676,401	rs2890652	LRP1B	C/T	0.18	0.16	1.05	0.092	1.06	0.87	1.01
3	85,966,840	rs13078807	CADM2	G/A	0.22	0.011	1.09	0.016	1.08	0.092	1.07
3	187,317,193	rs9816226	SFRS10,ETV5, DGKG	T/A	0.8	0.13	1.05	0.12	1.06	0.72	0.99
4	44,877,284	rs10938397	GNPDA2	G/A	0.4	0.48	1.02	0.42	1.02	0.51	0.98
4	103,407,732	rs13107325	SLC39A8	T/C	0.02	0.009	1.30	0.01	1.30	0.13	1.19
5	75,050,998	rs2112347	FLJ35779	G/T	0.33	0.92	1.00	0.86	1.00	0.81	0.99
5	124,360,002	rs4836133*	ZNF608	A/C	0.45	0.375	0.97	0.44	0.98	0.30	0.97
6	34,410,847	rs206936	NUDT3	G/A	0.2	0.77	1.01	0.82	1.01	0.72	1.01
6	50,911,009	rs987237	TFAP2B	G/A	0.18	0.49	0.98	0.37	0.97	0.06	0.93
9	28,404,339	rs10968576	LRRN6C	G/A	0.32	0.16	1.04	0.19	1.04	0.64	1.02
11	8,561,169	rs4929949	RPL27A	C/T	0.54	0.62	0.99	0.71	0.99	0.25	0.97
11	27,682,562	rs10767664	LGR4, LIN7C, BDNF	A/T	0.83	0.17	0.95	0.31	0.96	0.028	0.91
11	47,619,625	rs10838738	MTCH2	G/A	0.36	0.015	0.93	0.012	0.93	**$8.5x10^{-5}$**	0.88
12	48,533,735	rs7138803	BCDIN3D, FAIM2	A/G	0.37	0.003	1.09	0.0058	1.08	0.46	1.02
13	26,918,180	rs4771122	MTIF3	A/G	0.77	0.36	0.97	0.36	0.97	0.66	0.98

						SMM		Adj. Sex		Adj. Sex+ BMI	
Chr	**Position**	**SNP**	**Nearest gene(s)**	**EA/OA**	**Freq.**	**P**	**OR**	**P**	**OR**	**P**	**OR**
14	29,584,863	rs11847697	PRKD1	T/C	0.05	0.69	1.03	0.97	1.00	0.75	0.98
14	79,006,717	rs10150332	NRXN3	C/T	0.21	0.24	1.04	0.29	1.04	0.98	1.00
15	65,873,892	rs2241423	MAP2K5	A/G	0.21	0.021	0.92	0.1	0.94	0.92	1.00
16	19,841,101	rs12444979	GPRC5B	C/T	0.11	0.58	0.98	0.51	0.97	0.85	1.01
16	28,793,160	rs7359397	SH2B1, ATP2A1	C/T	0.43	0.16	1.04	0.12	1.05	0.7	1.01
16	52,361,075	rs1558902	FTO	A/T	0.42	**9.0×10^{-4}**	1.10	0.0031	1.09	0.76	0.99
18	55,990,749	rs571312	MC4R	A/C	0.25	0.1	1.05	0.16	1.05	0.73	0.99
19	39,001,372	rs29941	CHST8, KCTD15	A/G	0.69	0.15	1.05	0.14	1.05	0.99	1.00
19	50,894,012	rs2287019	QPCTL	C/T	0.21	0.2	0.96	0.16	0.95	0.85	0.99
19	52,260,843	rs3810291	TMEM160	A/G	0.68	0.19	1.04	0.21	1.04	0.86	1.01

Effect allele (EA) defined as the BMI increasing allele, and other allele (OA). P value adjusted for relatedness using inflation factors. This association was based on the imputation data from up to 3,142 SMM OSA patients and 40,664 controls with known BMI measurements, except for rs4836133*, which was based on imputation data from up to 3,069 SMM OSA patients and 24,125 controls with known BMI measurements. Significant p-values after Bonferroni adjustment (P<0.05/32=0.0016) are shown in bold.

Table 9. Association of the obesity risk variant rs10838738[G] in the MTCH2 gene at 11p11 with OSA and subgroups of OSA in the Icelandic and Australian sample sets.

		Adj. Sex		Adj. Sex + BMI	
Group	n_a/n_c[a]	P[b]	**OR (95% CI)**	P[b]	**OR (95% CI)**
SMM					
Iceland	3,094/25,724	0.0023	0.910	2.9×10^{-5}	0.873
Australia	1,627/4,893	0.054	0.920	0.044	0.908
Combined		0.00032	0.913 (0.869-0.960)	4.4×10^{-6}	0.884 (0.838-0.932)
Moderate/Severe					
Iceland	2,647/25,724	0.0044	0.911	2.7×10^{-5}	0.873
Australia	1,078/4,893	0.14	0.927	0.17	0.922
Combined		0.0014	0.916 (0.867-0.966)	1.4×10^{-5}	0.884 (0.836-0.935)
Males only					
Iceland	2,234/8,130	0.005	0.901	6.6×10^{-5}	0.853
Australia	1,008/2,097	0.45	0.958	0.42	0.950
Combined		0.0057	0.918 (0.863-0.975)	0.00014	0.879 (0.823-0.939)
Females only					
Iceland	860/17,594	0.17	0.931	0.090	0.913
Australia	619/2,796	0.039	0.873	0.033	0.856
Combined		0.03	0.922 (0.857-0.992)	0.0086	0.893 (0.820-0.971)
Obese					
Iceland	1,880/5,549	0.007	0.891	0.0040	0.881
Australia	865/841	0.036	0.862	0.065	0.869
Combined		0.00072	0.882 (0.820-0.949)	0.00056	0.876 (0.812-0.944)
Non Obese					
Iceland	1,214/20,175	0.0094	0.889	0.0038	0.877
Australia	762/4,052	0.22	0.930	0.24	0.932
Combined		0.0054	0.904 (0.843-0.971)	0.0029	0.898 (0.837-0.964)

[a]Number of OSA cases (n_a) and matched controls (n_c) genotyped on Illumina bead chips or Centaurus singletrack assays. [b]The Icelandic *P* values have been adjusted for relatedness by dividing the χ^2 statistic by 1.088, 1.069 (SMM, severe/moderate/mild), 1.074, 1.059 (Moderate/Severe), 1.077, 1.063 (males), 1.028, 1.013 (females), 1.028, 1.037 (obese) and 1.038, 1.036 (non-obese) in the analysis adjusted for sex and for sex and BMI, respectively.

Table 10. Comparison of the frequency of the obesity risk variant rs10828738-G in MTCH2 at 11p11 between different sub-groups of OSA cases.

rs10838738-G	Dataset	n_a/n_b[a]	f_a/f_b[b]	P[c]	OR
Analysis					
Moderate/Severe vs. Mild	Iceland	2647/447	0.343/0.341	0.9	1.009
	Australia	1078/549	0.342/0.337	0.78	1.022
	Combined			0.78	1.015
Males vs. Females	Iceland	2234/860	0.342/0.343	0.94	0.995
	Australia	1008/619	0.347/0.330	0.31	1.080
	Combined			0.57	1.027
Obese vs. Non-obese	Iceland	1880/1214	0.349/0.333	0.2	1.073
	Australia	865/762	0.342/0.339	0.89	1.010
	Combined			0.27	1.051

[a]Number of individuals in group a (n_a) and group b (n_b) genotyped on Illumina bead chips or Centaurus singletrack assays. [b]Frequency of variant in group a (f_a) and group b (f_b). [c]*P*-values for analysis of obese vs. non-obese individuals in Iceland adjusted for relatedness with the genomic control factor $\lambda_g = 1.028$. No adjustment was needed for the analysis of the other groups ($\lambda_g = 1$). Moderate/Severe : AHI and/or ODI ≥ 15, Mild: AHI and/or ODI ≥5 and <15

4.4 OSA candidate genes

Thirteen genes have been reported to show significant association with OSA (63, 116-127).

Variants in these genes and 50 kb around them, were tested for association in the main OSA phenotypes (all OSA patients, SMM and SM patients, as well as for the BMI subsets with BMI under and over 30). The significance threshold was based on the Bonferroni correction for the number of SNPs in each region.

For these 13 regions, no variants in the genes tested showed association with any of the phenotypes. When the region around each gene was expanded up to 50 kb around them one SNP (rs3852860) close to the ApoE gene showed association with moderate risk to obese SMM OSA patients $P=2.29\times10^{-5}$, OR=1.15 (significant p-value for this region 2.43×10^{-5}) (See Appendix). It is common (frequency >40%) intronic SNP located almost 30 kb upstream of the ApoE gene, in a gene called PVRL2 (encoding poliovirus receptor-related 2). When adjusted for BMI the association was no longer significant (P=0.001654, OR=1.14).

5 Discussion

The results of this study demonstrate that OSA aggregates in families, as shown earlier, but no genes or loci were found to be significanlly linked to OSA. Furthermore, the effect of obesity-related sequence variants on the risk of OSA was studied. One obesity-related sequence variant was shown to decrease the risk of OSA, i.e. opposite to its increased risk on BMI as previously published, and replicated in an Australian dataset.

5.1 Familiality

This study, based on over 4400 Icelandic OSA patients, confirms that OSA segregates in families, indicating common risk factors. Relatives of OSA patients are significantly more likely to have OSA than the general population. The risk for a first degree relative of having OSA is more than twofold and decreases for each degree relation; however it is still significantly above 1.0, even for distant relatives (third- to fifth-degree relatives).

This study confirms results from earlier studies on familiality of OSA. Familiality of OSA has been demonstrated in Iceland, partly based on the same individuals as in the current study (Icelandic OSA patients diagnosed before 1999) (52), the United States (35, 128), Scotland (36) and Israel (129) with risk of 1.5-2.0 for first degree relatives of OSA patients, which was slightly lower than in the current study.

The effect of obesity was tested by analysing the obese and non-obese OSA patients separately. Relatives of non-obese OSA patients have a similar risk as the relatives of all OSA patients. Obesity resulted in increased risk of sleep apnea, most pronounced for first-degree relatives of obese OSA patients that have a higher risk than more distant relatives (third- to fifth-degree relatives). The RR for first-degree relatives of obese OSA patients was higher than the RR of first-degree relatives of non-obese OSA, indicating that obesity adds to the familial risk. The same was true for other degrees of relatedness.

Spouses showed increased risk of having OSA, especially spouses of obese OSA patients, which indicates the effect of obesity on the risk of OSA. It is also possible that more awareness of OSA among the patient's spouses may explains the increased risk of OSA. It should be noted that the confidence interval is much wider for the spouses compared to relatives of OSA patients, due to the low number of spouses.

5.2 Linkage

In this comprehensive linkage analysis of OSA phenotypes none of the linkage peaks identified reached genome wide significance. Ten linkage peaks with LOD scores of >1.5 were observed (at 2q36, 5p14, 7p14, 7p21, 10q26, 11p14, 11q13, 11q25, 12q24, 19p13) and could be considered suggestive. The linkage peak at 5p14 was found for the main OSA phenotypes, i.e. before dividing them according to

BMI, whereas the peak at 11q25 was shared between OSA and obese OSA phenotypes. Most of the linkage peaks were confined to obese OSA phenotypes (at 2q36, 7p21, 10q26, 11p14, 11q13, 11q25), and three linkage peaks were confined to non-obese OSA phenotypes (at 7p14, 12q24, 19p13).

The linkage analyses were not adjusted for BMI but performed on high or low BMI subsets of OSA, in addition to the main phenotypes. Around 66% of the OSA patients had a BMI ≥ 30 and the linkage regions that were observed for obese OSA patients could be linked to obesity rather than sleep apnea itself. The highest LOD score was observed for a region at 11p14, which harbours the obesity gene *BDNF* (Brain-derived neurotrophic factor) (66) and a region at 11q13, where a suggestive linkage to BMI-defined obesity has been reported (130). It harbors two genes, *UCP2* (mitochondrial uncoupling protein 2) and *UCP3* (mitochondrial uncoupling protein 3) that have been linked to obesity (131, 132).

Linkage peaks are often broad and harbour many genes. In attempt to further analyse the signal the suggestive linkage peaks were finemapped using SNP genotypes from the Illumina chip to search for significant associations with OSA compared to population controls. Available SNP chip data for 2,148 SMM OSA patients and >36,000 controls were used. After adjusting for the number of SNPs tested for each linage peak with the Bonferroni correction, only one sequence variant, rs681566 in the NEU3 gene at 11q13, showed a significant association with obese SMM OSA. The association was further tested in additional Icelandic OSA patients and the European-American OSA patients but did not replicate. It was therefore concluded that this association was unlikely to be real.

There were three linkage peaks at 7p14, 11p14 and 19p13 that overlap with chromosomal regions previously published as linking with OSA (61). At chromosome 7, a LOD score of ~1.5 was observed around 50 cM, but was only seen for BMI (both adjusted and un-adjusted for AHI) (61), compared to the LOD score of 1.74 found at 62 cM that was seen for non-obese OSA patients in Iceland. At chromosome 11 around 40 cM, a LOD score of 2 was seen for AHI unadjusted for BMI and a LOD score around 1.5 for BMI unadjusted for AHI (61). In Iceland this region gave LOD score of 2.4 for obese SM OSA patients. At chromosome 19 at ~40 cM, a LOD score of 2 was seen for AHI unadjusted for BMI in European Americans (61) compared to a LOD score of 2 in non-obese Icelandic OSA patients. However, as stated above, finemapping of these regions did not reveal a significant association with OSA in Iceland and no attempt was made to finemap these regions in the previous study (62) apart from the ApoE region (61).

Apart from these three regions, no other linkage peaks overlaped between these three studies (61, 133). There can be several reasons for this. In previous studies the AHI score was used as a continuous variable, and the data were analyzed with and without adjusting for BMI (61, 87, 133), whereas in the current study patients diagnosed with OSA were grouped according to severity (CPAP users, severe, moderate and mild) and subsets with BMI under or over 30 were analysed as well. By analysing obese and non-obese OSA patients separately rather than adjusting for BMI, we might lose power to detect linkage peaks. However, the BMI subsets were larger (1,790 obese OSA and 1,220 non-obese OSA patients) than the sample sets in previously published studies (N=349 Caucasians (60), N=641 Caucasians (61)). In addition the Icelandic cohort was based on patients diagnosed at hospitals and undergoing treatments whereas the cohorts in previous studies were population based.

5.3 Obesity and OSA

When sequence variants recently found through large genome-wide association studies to associate with obesity (66, 90, 94) were tested for association with OSA, with and without adjusting for BMI, two SNPs were found to associate with OSA; one variant at 16q12 in the obesity-related *FTO* gene (66, 88), and another at 11p11 in the *MTCH2* gene.

The variant in the *FTO* gene, rs1558902, is located in the first intron of the gene and associates with increased risk of OSA. Of the 32 variants tested in this study, rs1558902 showed the strongest association with BMI (94); hence it is not unexpected that it also associates with OSA. The association disappeared when adjusted for BMI, indicating that its effect on OSA is mediated through obesity. The *FTO* gene is widely expressed in the brain (88, 134). Loss of *FTO* expression reduces adiposity, but the true function of the gene is not clear. A knockout study showed that *FTO* controls energy expenditure, indicating that leanness is a consequence of increased energy expenditure (97). In an obesity study about 16% of the population were homozygous for the risk allele of a variant (rs9939609) that is correlated with rs1558902 (r^2=0.90) and do weigh on average 3 kilograms more than non-carriers (88).

The G allele of rs10838738 in the *MTCH2* gene was previously shown to associate with higher BMI (90). In the current study it was found to associate with *reduced* risk of OSA, first in the Icelandic sample set and then supported by the Australian sample. When adjusting for BMI the association was stronger. This association was not affected by the severity of OSA, and there was no significant difference between men and women when adjusted for BMI. The MTCH2 gene is strongly expressed in the liver and at low levels in the brain (90). *MTCH2* encodes a carrier protein involved in transport in mitochondria and MTCH2, in complex with met-induced mitochondrial protein (encoded by MIMP), has a critical function in apoptosis through the tBID-BAX (truncated BH3-interacting domain death agonist-apoptosis regulator BAX) death pathway by regulating the recruitment of tBID to mitochondria (135, 136). The variant rs10838738 is non-coding and it is not clear whether it affects expression of MTCH2 or how it contributes to the pathogenesis of OSA, although our results suggest that its effect is not through obesity- related pathways.

5.4 OSA candidate genes

Thirteen genes have been reported to show significant association with OSA (63, 116-127). No variants in these genes showed association with OSA in the main OSA phenotypes (all OSA patients, SMM and SM patients, as well as for the BMI subsets with BMI under and over 30). When the region around each gene was expanded up to 50 kb around them one SNP showed association with obese SMM OSA patients but when adjusted for BMI the association was no longer significant.

These candidate gene studies have been performed on different OSA phenotypes of various ethnic backgrounds. Most of these studies were small, based on 30-1,775 cases, often with weak results. When studies based on few individuals are reported and statistically criteria are not stringent enough, the chances of false positive results increases. The lack of association with risk to OSA in this study,

which was based on a large and well phenotyped OSA cohort, indicates that previous studies reporting the risk of OSA (63, 116-127) are unlikely to be true.

5.5 Future research

Today 7,583 individuals have been diagnosed with OSA in Iceland and of them, 3,670 have undergone sleep studies. Around 70% of these individuals have been genotyped on any of the Illumina Beadchips. Collection of OSA phenotypes and genotyping is still ongoing

At deCODE Genetics over 105,000 individuals have been typed on various Illumina chips, and in 2010 a large whole-genome sequencing project started using Illumina GAIIx and HiSeq2000. More than 2,600 Icelanders have been sequenced to a depth of at least 10x (average 20x), and based on their sequence data around 40 million markers (SNPs and indels) have been imputed into the Icelandic population and partially into additional 280,000 individuals.

By using previously described methods (67, 137) it is possible to perform a long-range phasing of haplotypes (138) on all individuals that have been genotyped on Illumina chips. SNPs that are identified through sequencing have been imputed into all Icelanders who have been phased using IMPUTE (137). In addition genotypes can partially be imputed into relatives of chip-genotyped individuals, using the fully phased genotypes available. After phasing and imputation, association analysis is performed with logistic regression, matching controls to cases based on the information score of the imputed genotypes (67, 69).

Association analyses are performed regularly for all the OSA phenotypes described above as well as for sleep related traits like hours of sleep, cataplexy, bruxism, sleep talking and sleep walking. With increasing genotyping and phenotype information it is expected that sequence variants that contribute to the risk of OSA and sleep-related traits will be identified allowing us to determine the genes and pathways that play a major role in the pathogenesis of OSA and related traits.

6 Conclusions

Relatives of OSA patients are more likely to have OSA than the general population, and obesity adds to the risk. However, non-obese OSA patients also show familial aggregation indicating that risk factors other than obesity play a role.

Linkage studies of OSA have not been successful and no linkage peaks reached genome-wide significance in our study. Finemapping of suggestive linkage regions with a high density of SNPs did not reveal significant associations. Overall linkage analysis has been an unsuccessful approach for discovery of sequence variants that contribute to the risk of obstructive sleep apnea or severity of the condition.

We identified a sequence variant in the *MTCH2* gene at 11p11 that significantly associates with reduced risk of OSA, and mediates its effect on OSA through non-obesity related pathways. Sequence variants in the *FTO* gene at 16q12 confer risk for OSA through obesity-related pathways.

Analyses of variants in proposed candidate genes for OSA did not show association with any of the OSA phenotypes tested. When each region was expanded, one SNP in the gene PVRL2 showed significant association with obese SMM OSA patients. This variant is located almost 30 kb upstream of the candidate gene ApoE. Further analysis is needed to determine if this signal is real.

OSA is a complex trait with complex etiology and many potential contributing factors. One advantage of this study has been the use of the Icelandic sleep apnea cohort (ISAC) which is large and well phenotyped. It is a unique source for further genetic studies, well suited to discover the association of common and rare sequence variants with small or large effects on OSA phenotypes. Further studies based on genome-wide association and whole genome sequencing will hopefully reveal sequence variants that confer the risk of OSA and help us to understand the mechanisms involved.

7 References

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Table: Genes previously reported to associate with OSA tested in the Icelandic OSA cohort.

Gene	Gene region +/- 50 kb	Number of SNPs	lowest observed p-value	OR	SNP	Freq. Iceland	Phenotype	ref
ACE	chr17:58858154-58979473	1,626	$4.25x10^{-4}$	3.06	chr17:58979087	0.12	SM	(117, 118)
APOE	chr19:50050879-50154490	2,061	**$2.29\ x10^{-5}$**	**1.15**	**rs3852860**	40.36	SMM w BMI≥30	(119)
ADRB2	chr5:148136349-148238390	1,345	$1.09\ x10^{-4}$	0.24	chr5:148219391	0.49	SMM w BMI≥30	(120)
CRP	chr1:157898703-158001003	1,222	$2.28\ x10^{-3}$	1.10	rs4656849	39.38	SM	(63)
CYBA	chr16:87187198-87294958	2,053	$6.61\ x10^{-4}$	0.01	chr16:87200298	1.65	SM	(116)
EDN1	chr6:12348515-12455413	1,481	$2.28\ x10^{-4}$	1.81	chr6:12438250	0.61	SM	(121)
EDNRA	chr4:148571519-148735556	2,245	$2.29\ x10^{-4}$	3.00	chr4:148703738	0.18	SMM w BMI≥30	(122)
GDNF	chr5:37798536-37925539	1,747	$6.76\ x10^{-4}$	1.42	rs115745064	1.85	OSA all w BMI≥30	(63)
HP	chr16:70596009-70702456	1,461	$8.71x10^{-5}$	1.16	rs8051882	34.58	SM w BMI≥30	(123)
IL6	chr7:22683291-22788146	1,618	$5.43\ x10^{-4}$	1.31	rs118166722	1.76	OSA all	(124)
LEPR	chr1:65608923-65925764	4,426	$1.22\ x10^{-4}$	2.05	rs189350034	0.52	SMM w BMI≥30	(125)
TNF	chr6:31601329-31704091	1,883	$2.20\ x10^{-4}$	1.62	rs986476	1.01	OSA all w BMI≥30	(126)
SLC6A4	chr17:25497504-25637080	1,811	5.33E-04	0.393	chr17:25605730	0.29	OSA all	(127)

For each region tested (build 36), the number of SNPs within each region and the lowest observed p-value is shown. The best SNP for each region with p-value and odds ratio (OR) is reported as well as the frequency in Iceland and the phenotype the association was with. The thresholds for significant p-values were found with the Bonferroni correction based on number of SNPs tested in each region. Significant p-value is shown in bold. Data are based on imputation of up to 7,505 OSA patients and 78,045 controls.