Genetics of sleep disordered breathing (syndromes excluded)

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SUMMARY
The objective of this article is to present the approach, the main methods and the main results obtained in the study of the genetics of sleep disordered breathing. It is not an exhaustive systematic review of the literature but a didactic presentation to aid understanding of current research in the domain. It highlights the existence of a genetic predisposition to developing respiratory sleep disorder, from simple snoring to sleep apnea syndrome. The genes and pathways most frequently identified are those involved in inflammation.

KEYWORDS
Sleep disordered breathing, OSAHS, genetic predisposition, risk factors, inflammation

INTRODUCTION
There is a continuum of sleep disordered breathing, from snoring to apnea. A large part of the population are affected, increasingly with age and overweight/obesity, and more frequently males (20%) than females (10%)¹. Although mostly studied in adults, respiratory sleep disorder also affects children (prevalence, 1% to 4%)². Sleep disordered breathing consists in iterative partial or complete obstruction of the upper airway³. Definitions and diagnostic criteria have evolved over time, but are still founded on polysomnography (PSG) or respiratory polygraphy (RP) and the respiratory disturbance index (RDI). In epidemiology, these are seldom available, and studies have, so far, been mainly based on questionnaires and/or sleep diaries.

Several steps are needed to analyze genetic predisposition to a phenotypic trait, such as sleep disordered breathing, in humans. The first is to demonstrate a familial component: i.e., that certain families show abnormal numbers of cases. This is achieved by familial aggregation studies. The second is to demonstrate a genetic component within this familial component. This is mainly achieved by twin studies. When the phenotype is a common one (as in the present case), genetic study uses genetic epidemiology, based on large samples (of families, of populations of several hundreds), using statistical methods, exploring for variations in markers or common genetic polymorphisms exerting moderate effects. The term here is “complex genetic predisposition” rather than Mendelian genetic predisposition, which identifies genetic mutations or abnormalities that are generally rare but exert a strong deleterious effect, with an equally rare but severe phenotype (e.g., achondroplasia).
The study of complex genetic predisposition has progressed since the 1990s, with the development of typing methods (of genes or gene expression), which have become increasingly available, technologically, methodologically and financially (Fig. 1). For about 10 years, study of gene expression (messenger RNA (mRNA) measurement) and epigenetics (transcriptional modification without modification of DNA) or genome-wide association studies (GWAS) and/or direct whole-genome sequencing have been developing. Very recently, studies of networks or systems combining several such “omic” analyses have begun to be published.

FAMILIAL COMPONENT

Familial aggregation

There have been several reports of families with high incidence of sleep disordered breathing over several generations.

For example, El Bayadi et al., in the United States in 1990, reported a family of 9 spanning 3 generations recruited via an index case, a 55 year-old patient with severe snoring and apnea (apnea/hypopnea index (AHI) > 30/h), non-restorative sleep and excessive daytime somnolence. Nine of the index case’s siblings, children and grandchildren were habitual snorers with non-restorative sleep, 5 showed daytime somnolence, and 3 snored and showed sleep apnea. None showed retrognathia; 3 had a 22-32 mm mandibular plane - hyoid bone distance (MPH); 4 had 7-9 mm pharyngeal airway space (PAS); and 5 were overweight (BMI, 25-30 kg/m²). On RP, AHI was 15-30 in 4 cases, 5-15 in 3, including the grandson, and normal in only 1. This study thus shows familial aggregation of severe snoring, independently of BMI and more or less independently of cephalometry.

In 1995, Guilleminault et al. reported on 3 American families of 3 generations, with children aged 3-16 years, diagnosed with sleep apnea syndrome (OSAS) confirmed on PSG and RDI > 5/h. Sixteen of the 27 subjects examined and explored were diagnosed with obstructive sleep apnea syndrome (OSAS), including 7 children aged 3-16 years. Clinical examination found deep ogival palate and retropositioned mandible suggesting cephalometric abnormality.

Sundquist et al., in a siblings study, assessed the risk of hospital admission for OSAS in Swedish adults aged 19-72 years when an elder sibling had been admitted for OSAS. A total of 12,763 male and 3,037 female subjects were included from Swedish registries. Standardized incidence ratios were calculated, distinguishing men and women, and, overall, proved on average ratios to be increased by a factor of just over 3, regardless of gender.

They also found two peaks for age at diagnosis in male or female patients with familial history of OSAS.
compared to those without: at 30-34 and 60-64 years, both more pronounced in men than women. They found no correlation between spouses, suggesting little impact of adult environment. Given the origin of the data, no clinical details were available (notably, BMI).

These studies thus show familial aggregation of respiratory sleep disorder, involving several generations, including children.

Some also suggest an impact of facial development and BMI, although without explaining all of the cases described. Such familial aggregation may represent a shared environmental component and/or a genetic component.

**Twin studies**

It is generally agreed that, for monozygotic twins (identical twins who developed from a single ovum), phenotypic concordance is due to shared genetic inheritance. In dizygotic twins, who developed from two separate ova, phenotypic concordance is due only to the half of the genotype they share. Presence of a genetic component is suggested when phenotypic concordance is stronger in mono- than di-zygotic twins.

In the USA, Carmelli et al. (2001) studied 1,560 pairs of male twins with a mean age of 74 years and found 24% concordance for habitual snoring in monozygotic twins (818 pairs) compared to only 9% in dizygotic twins (742 pairs). Likewise, Desai et al., in the UK in 2004, studying 1,937 pairs of female twins with a mean age of 51 years (range, 20-80 years), found 28% concordance for snoring disturbing the sleep of others and/or awakening them frequently or systematically in monozygotic twins (933 pairs) versus...
14% in dizygotic twins (1,004 pairs). In a more refined exploration of the twin pairs, Carmelli et al. focused on the coexistence of habitual snoring and obesity versus presence of only one or the other phenotype in their twin population, 29% of whom had BMI of at least 28 kg/m². They found 13% correlation between obesity and snoring, entirely attributable to shared genetic factors. They further estimated that only 30% of the genetic component in snoring could be attributed to genes also underlying obesity. Individual heritability of snoring was thus estimated at 23% (95% confidence interval (CI): 18%, 28%).

They thus suggested that snoring in elderly males has a familial basis with a genetic component that is largely independent of genes related to obesity. Likewise, Desai et al., modeling a genetic effect as co-dominant with an environmental effect in their female twin pairs, estimated raw heritability of disturbing snoring at 52% (95% CI:36%, 68%), falling to 42% after adjustment on BMI, smoking and menopausal status.

Taken together, these studies demonstrate multigenerational familial aggregation of sleep disordered breathing, in different countries: i.e., in populations of differing origins (Caucasian, African American or Asian). They further demonstrated that this familial component was partly accounted for by genetic factors, and estimated heritability at 21% to 84% depending on population, phenotype (type of disorder studied) and associated factors such as BMI or morphology.

**FAMILY STUDIES**

Historically, the first analyses of genetic predisposition were family studies: (i) familial segregation studies seeking to identify a so-called major gene with Mendelian transmission (dominant, co-dominant or recessive) within multigenerational families, or (ii) linkage studies seeking to locate a genetic effect, either on models derived from segregation analyses of multigenerational families or directly from data, usually concerning siblings, collected without preconception regarding the underlying genetic model of transmission.

**Segregation analyses**

The principle consists in comparing the distribution of the observed phenotype (sleep disordered breathing) in the studied population versus the expected distribution according to various Mendelian models (dominant, co-dominant or recessive), taking account of known risk factors (gender, BMI, etc.), familial resemblance (parent-child, sib-sib, etc.) and the characteristics of the major gene being modeled (frequency of “at-risk” allele, and probability of being affected according to genotype: i.e., penetrance). These analyses are purely statistical and require no genetic typing. Two such studies have been published.

Holberg et al. studied snoring using a self-administered questionnaire between 1972 and 1996 in 584 families totaling 2,019 non-Hispanic Caucasian individuals aged 10 years and
over\textsuperscript{9}. They focused on two phenomena: isolated snoring, and snoring associated with daytime somnolence as proxy for obstructive sleep apnea/hypopnea syndrome (OSAHS). They also took account of gender, age and BMI as risk factors. In isolated snoring, they found a significant familial resemblance between mothers and children and between siblings but not between spouses who present no genetic heritage in common. They found no major gene effect, and suggested a polygenic effect with several genes involved, none of which predominated, and/or shared environmental effects. In OSAHS, they found significant resemblances between parents and daughters, especially as regards mothers, pointing to a major dominant or co-dominant gene (either being compatible) with residual familial correlations suggestive of other unidentified genetic and/or environmental effects.

Buxbaum \textit{et al.} studied 177 Caucasian families (1,195 individuals) and 125 African American families (720 individuals), with ages between 2 and 85 years, from the \textit{Cleveland Familial Study} cohort (1990-1993)\textsuperscript{10}. They focused on AHI measured at home by RP, defined as at least 10 seconds’ reduction in or arrest of airflow and at least 2.5% reduction in oxygen saturation. They found familial resemblance between 1\textsuperscript{st} degree relatives (parent-child, siblings) but not spouses, thus suggesting a genetic effect. In Caucasians, they found a recessive major gene after adjustment on age, explaining 28% of the variance. Adjustment on age and BMI eliminated the genetic effect in males, suggesting a major role of BMI. In African American families, they found a co-dominant major gene, accounting for 35% of variance; adjustment on BMI enhanced this genetic effect.

These two studies demonstrated genetic heterogeneity: several genes seem to be involved, some exerting a Mendelian effect (major genes), the genes and associated factors such as BMI varying according to population.

\textbf{Linkage analysis}

Once a genetic model has been established, the major gene has to be located within the genome. This is achieved by genetic linkage analysis, using parameters estimated on segregation analysis. Linkage analysis involves typing genetic polymorphisms using dedicated or non-dedicated chips, and thus requires DNA from family members. One or more regions of the genome are then explored for genes which are transmitted within the family in the same way as the gene characterized by the parameters estimated on segregation analysis. Linkage is measured by LOD (logarithm of odds) score. No such studies have been published regarding snoring or OSAHS.

Another approach involves linkage analysis without presuppositions as to the genetic model underlying transmission. In the case of pairs of siblings, for example, for each polymorphism, the proportion of shared alleles that are identical by descent (IBD: identical to those of the parents) is compared to the proportion expected on Mendelian grounds (Fig. 2): there is a genetic relation when there is an excess of IBD alleles shared by siblings presenting...
the same phenotype. Palmer et al. and Larkin et al. performed such analyses in the above-mentioned Cleveland population. They obtained genotyping data for 641 Caucasians (109 families) and 634 African Americans (128 families). They identified several regions with LOD scores suggesting linkage with AHI and/or OSAHS. In Caucasians, the main peaks for AHI were on chromosomes 6 and 10 in 6q23-25 and 10q24-q25, respectively. These two peaks, which diminish after adjustment for BMI, suggest a pleiotropic effect of these regions. Another promising region was in 6p11-q11, close to the orexin-2 receptor gene, suggesting passage via a cascade independent of BMI. In African Americans, the strongest links with AHI after adjustment on BMI were in 8p21.3, increasing after adjustment on BMI, and in 8q24.1, decreasing after adjustment on BMI. These analyses suggest that there are genetic regions related to sleep disordered breathing, acting both independently of BMI and by pathways linked to BMI.

Relf et al. performed linkage analysis in a Filipino family of 50 (50% adults), recruited via a child presenting with OSAHS diagnosed by PSG. The family showed high prevalence of OSAHS and metabolic abnormalities: 46% of members (9 adults, 14 children) had OSAHS, severe in 5 cases (4 adults, 1 child). Moreover, at least two metabolic abnormalities (obesity, type-2 diabetes, hypertriglyceridemia, insulin resistance, or HDL or total cholesterol elevation) were found in 48% of the children. Linkage analysis found a significant peak in 19q13.4 for both OSAHS and HDL cholesterol level. Several candidate genes are present in this linkage region, including NK cell immunoglobulin receptor genes involved in modulating inflammatory response to cellular stress and to onset of atheroma.

The next step comprises case-control or association studies on candidate genes identified within the linkage regions.

**Figure 2**

Diagram of linkage analysis without presuppositions on the underlying genetic model of transmission, based on sibling pairs.
**CASE-CONTROL STUDIES**

**Probable candidate genes**

Case-control studies on probable candidate genes compare polymorphism frequency in candidate genes identified on linkage analysis or by prior knowledge found in the literature. To the best of our knowledge, no case-control studies have been published following the above-mentioned linkage studies. On the other hand, there have been several based on the literature, focusing on genes involved in inflammation and/or immunity\textsuperscript{15-22}, metabolism\textsuperscript{15-17,23-25}, cardiovascular disease\textsuperscript{16-18,26-28}, or neurological and sleep disorder\textsuperscript{16,19-21,29-32}. Figure 3 shows the overlap between some of these fields.

**GWAS**

For about the last 10 years, genotyping techniques and costs have greatly changed, allowing whole-genome case-control studies, known as *Genome-Wide Association Studies*: GWASs. In sleep disordered breathing, a consortium was set up to centralize data on Caucasians and African Americans: the CARe consortium. A dedicated chip, comprising genes targeting the heart, lungs, blood and sleep, was used, including slightly more than 45,000 polymorphisms. As the study included several populations from different cohorts, a meta-analysis approach was adopted. Two phenotypes were focused on: AHI

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![Figure 3](image_url)

*Main genes associated with sleep disorder breathing in case-control studies. In bold, genes replicated by different teams. #: results for both adults and children. In grey, associated factors taken into account or not according to the study.*
and OSAS diagnosed on PSG or RP (AHI ≥ 15/h). For Caucasians, the main analysis concerned 2,904 individuals (664 from the Cleveland study, 1,673 from the Atherosclerosis Risk in the Community (ARIC) study, and 567 from the Framingham study). For AHI, there were no significant findings (with p < 10^−6). However, the polymorphism with the smallest p-value lay on chromosome 9 in a non-coding region of the LPAR1 gene, which codes for a protein with a pro-inflammatory role promoting circulating monocytes and also known to be associated with neurologic and craniofacial abnormalities in mice. Regarding OSAHS, analysis revealed a significantly associated polymorphism on chromosome 1 in a non-coding region of PTGER3, with enhanced association after adjustment on BMI. PTGER3 codes for a protein modulating central and peripheral neurotransmitter release. It is also part of a haplotype (group of polymorphisms transmitted together) associated with onset of high blood pressure. The polymorphism with the next lowest p-value was the one observed on chromosome 9 and associated with the AH1 phenotype.

For African Americans, the main analysis concerned 647 individuals from the Cleveland cohort. Analysis found a significant association (p < 10^−6) between AHI and the chromosome 9 polymorphism in the non-coding region of LPAR1, enhanced in non-obese subjects, and an almost significant association with a polymorphism in a non-coding region of ITPR2, the corresponding protein playing a role in intracellular calcium regulation and being implicated in inflammation, endothelial dysfunction and blood-pressure abnormalities. There was also a significant negative association in non-obese subjects between OSAHS and a polymorphism lying close to the PLEK gene, coding for a protein involved in actin assembly within leukocytes and thus contributing to exocytosis. The third polymorphism identified, although non-significant, concerned the LPAR1 gene on chromosome 9.

The next step was to replicate these results in similar populations on a case-control study of candidate polymorphisms. Three were selected, but only two could be adequately typed (LPAR1 on chromosome 9, and PTGER3 on chromosome 1), in 1,795 Caucasians taken from the Western Australia Sleep Health Study (WASHS) and 1,010 African Americans taken from the Cleveland study (n = 459) and the Case Transdisciplinary Research in Energetics and Cancer Colon Polyps Study (CTRECCPS) (n = 551). These replication populations differed from the primary populations in terms of prevalence of OSAHS: the Caucasian population included more males (63% versus 47%) and obese subjects (mean BMI, 33 versus 29 kg/m^2), with a higher prevalence of OSAHS (77% versus 33%); the African American population was composed of two very different populations with female predominance (78% and 67%), one being a female OSAHS cohort with BMI of 41 versus 32 kg/m^2 (n = 459), and the other being a female cohort with BMI of 31 kg/m2, free of sleep disordered breathing (n = 551). The association between AHI and the PTGER3 polymorphism on chromosome 1 on a co-dominant model was
replicated in Caucasians, as well as the association between OSAHS and the \textit{LPAR1} polymorphism on chromosome 9 in African Americans. These two findings confirm that a complex genetic predisposition is involved in sleep disordered breathing. The two polymorphisms concern genes implicated in inflammation, raising the question of causality. Moreover, taking BMI into account seems to make little or no difference, suggesting that the effects are independent. Functional studies will be needed to establish the causal role of these polymorphisms and to understand the mechanisms involved, but have yet to be performed and/or published.

**Gene expression (messenger RNA)**

**Studies based on candidate gene transcription (with prior knowledge)**

A recent functional study by David Gozal’s team\textsuperscript{18} included 605 children aged 5-10 years, with or without OSAHS, and concerned polymorphisms of a candidate gene, \textit{EDN1}, coding for a protein involved in an intracellular signal implicated in vasoconstriction, left ventricle hypertrophy and asthma\textsuperscript{38,39}. It was recently shown that four EDN1 polymorphisms are frequent in children with OSAHS. The functional study showed that EDN1 expression, assessed by cell quantity of specific messenger RNA (mRNA), was higher in children with OSAHS than in those without (mean, 1.8 \textit{versus} 1.65).

**Studies based on genome-wide transcription (without prior knowledge)**

Another approach is to explore whole-genome transcription, as was also done by David Gozal’s team\textsuperscript{40} in a case-control study in 40 non-obese children aged 4-10 years, the 20 case subjects having OSAHS on PSG, with tonsillar hypertrophy. The chip, containing 44,000 transcripts (mRNA) covering the whole genome, identified 68 transcripts from white blood cells with differential (over or under) expression in cases. They were thus able to identify biological cascades or pathways, including 23 cellular and 32 functional processes, among which inflammatory response predominated. These methods open up complementary lines of research on the mechanisms underlying biological and organic abnormalities, but require molecular and functional confirmation.

**Epigenetics**

Gene expression may be altered without change in the genetic sequence itself. Several mechanisms have been identified that increase or decrease gene expression: (i) histone modification: histones are protein groups which chromosomal DNA curls around; modification may consist in acetylation, methylation, phosphorylation or ubiquitination; (ii) direct DNA methylation; or (ii) micro-mRNA fixing on DNA. DNA methylation has been most widely studied.

David Gozal’s team performed a first case-control study in 5-10 year-olds matched for age, gender and ethnicity, with or without OSAHS on PSG\textsuperscript{41}. They studied DNA methylation in a candidate gene (\textit{FOXP3}) and its regulatory regions. The coded protein is involved in immune regulation\textsuperscript{42}. They demonstrated hypermethylation
in the FOXP3 promoter region, especially in case of CRP elevation, which is a marker for inflammation. They demonstrated hypermethylation of the eNOS promoter region in 4-12 year-olds with OSAHS associated with delayed post-occlusion response⁴³, inducing significant reduction in mRNA expression as compared to children without response delay, proportionally to post-occlusion response time.

Expression-based networks and systems

Information from several “omic” studies can be combined, allowing global network or system analysis, based on bioinformatics. One example is Liu et al.’s study⁴⁴, in which a network was created from candidate genes and/or proteins identified in the literature, taking account of genes and/or proteins involved in craniofacial morphology, obesity, inflammation, respiratory control and pleiotropic effects. Networking was weighted by the number of genes and proteins included and their inter-relations. Data from public data-bases on protein-protein interactions and adipose tissue sequencing were then included, along with gene expression data (relative mRNA quantification) from a case-control study of postoperative subcutaneous and visceral adipose tissue from adult patients with or without OSAHS. Frequency scores were then calculated for each protein according to the networks they belong to, ranking them in decreasing order. The most frequent proteins, whatever the network, were those mainly involved in inflammation, some of which had already been associated with OSAHS (e.g., PDGF, EDN1, NOS3) or associated disorders (e.g., PI3K, STAT, MAPK, associated with insulin resistance, inflammation, etc.).

CONCLUSIONS/PERSPECTIVES

Research into the genetics of sleep disordered breathing is progressing. Results so far demonstrate a complex genetic predisposition and point to involvement of specific genes according to phenotype (e.g., AHI or OSAHS, or isolated or associated OSAHS), ethnicity, presence of overweight/obesity, etc. Even so, most genes playing a role in whatever context seem to be part of pro-inflammatory cascades. The reports require confirmation, by replication or functional or other studies. However, to this end there remain many questions in suspense and methodological issues to resolve. Which phenotypes should be studied? Using what definitions? Should the focus be on early and/or severe forms, or take account of a fuller range of factors and associated disorders (BMI, craniofacial development, etc.)? Which populations should be studied? Specific sub-populations: children, at-risk ethnic groups? What sample size? Sample size is very important if “omic” methods are used,
so as to ensure sufficient detection power despite multiple testing. But how is it to be achieved? Should cohorts be shared in a consortium? If so, how to deal with heterogeneity of populations (gender, age, ethnicity) and of definitions of phenotypes of interest: identical diagnostic criteria across cohorts? Should the same complementary data be collected, using identical definitions and coding: BMI, craniofacial data, etc.? Are the genetic, epigenetic and protein typing methods identical or at least partially superimposable? There are indeed many chips on the market, and they are developing rapidly. Finally, what type of analysis is needed? Adjusted, stratified, matched? On which criteria? Should gene-gene and gene-environment interactions be studied? Should several genes be studied simultaneously (e.g., haplotypes)? Should approaches be combined, especially “omics”? What methods? What criteria?

A lot of work, then, remains to be done to shed light on the pathophysiology of sleep disordered breathing, and perhaps improve management.

Conflicts of interest: The authors declare no conflicts of interest.

REFERENCES