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REVIEW ARTICLE

The genetics of sleep apnea

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Obstructive sleep apnea hypopnea syndrome (OSAHS) is a complex chronic condition that is undoubtedly influenced by multiple factors. Accumulating data suggest that there are strong genetic underpinnings for this condition. It has been estimated that approximately 40% of the variance in the apnea hypopnea index (AHI) may be explained by familial factors. It is likely that genetic factors associated with craniofacial structure, body fat distribution and neural control of the upper airway muscles interact to produce the OSAHS phenotype. Although the role of specific genes that influence the development of OSAHS have not yet been identified, current research in rodents suggests that several genetic systems may be important. In this chapter, we shall first define the OSAHS phenotype, and then review the evidence that suggests an underlying genetic basis of OSAHS, the risk factors for OSAHS that may be inherited, and potential candidate genes.

Key words: genetic epidemiology, heritability, sleep apnea.

Introduction

Recently, there has been a marked increase in our appreciation that obstructive sleep apnea hypopnea syndrome (OSAHS) is not simply a disorder of obese males, or one which affects people with clear craniofacial abnormalities, but is a disorder that likely affects a large proportion of the population. Furthermore, we now recognize that many of the key risk factors for the disorder have a large heritable component, and that OSAHS itself has a strong familial basis. It is likely that genetic factors associated with craniofacial structure, upper airway soft tissues, body fat distribution, neural control of the upper airway and central regulation of breathing interact to influence the expression of the disease. As the appreciation of the genetics of OSAHS progresses, it is likely that a greater understanding of fundamental pathophysiological mechanisms will follow, as will the development of more efficient approaches for identifying individuals at high risk for the disorder and likely to benefit from specific therapies. Although the role of specific genes that influence the development of OSAHS have not yet been identified, current research suggests that several genetic systems may

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influence the expression of the disorder. In this review, we shall first define the OSAHS phenotype, and then review the evidence that suggests an underlying genetic basis of OSAHS, the risk factors for OSAHS that may be inherited, and potential candidate genes.

Definition of the OSAHS phenotype

In addressing the genetics of OSAHS, we should first consider how best to define a clear, measurable phenotype. Ideally, an approach for phenotypic characterization would include measures that are reliable (i.e. consistent across days and across evaluators), accurate indicators of an underlying trait.

OSAHS is defined by a constellation of signs and symptoms; specifically, the occurrence of repetitive episodes of complete or partial obstruction of the upper airway during sleep, usually in association with loud snoring and daytime sleepiness [1]. Physiologically, such episodes are often associated with arousals, sleep fragmentation, intermittent hypoxemia and hypercapnia, and nocturnal hypertension [2].

The disease-defining metric that is commonly used in clinical and research settings is the apnea hypopnea index (AHI), which is simply a count of the number of episodes of breathing obstruction exceeding a critical duration, per hour of sleep. This index may be moderately correlated with various indices of night-time oxygen desaturation and sleep fragmentation [3]. Subjects are often considered "diseased" if the AHI, measured using overnight polysomnography, exceeds some threshold value. Alternatively, the AHI can be considered as a "continuous trait" (similar to blood pressure), with increasing values indicative of increasing disease severity. The advantages of using the AHI to classify disease status include its relative simplicity and high night-to-night reproducibility [4]. Most studies of the genetics of OSAHS have utilized the AHI as the major disease-defining variable, and these studies have demonstrated significant familial aggregation. The disadvantages of using the AHI as a sole indicator of disease include the between-laboratory variability in measurement technique, its lack of informativeness regarding the severity of individual events (duration, associated hypoxemia and arousal) and its uninformativeness regarding the functional and physiological impact of the disorder.

In 1998, an expert group was convened by the American Sleep Disorders Association to address the limitations of common approaches for defining OSAHS and to recommend approaches for standardization. The expert panel recommended approaches for using validated sensors and uniform measurement approaches as well as for combining data on daytime symptoms (e.g. sleepiness, vigilance), night-time symptoms (e.g. snoring, breathing pauses) and the AHI to classify disease status [5]. Such a system has yet to be validated and it is not yet clear whether this system distinguishes those with different long-term outcomes or those who differentially respond to therapy. Moreover, no study of the genetics of OSAHS has evaluated the heritability of the syndrome defined using the multiple dimensions recommended in this report.

OSAHS is undoubtedly a complex disorder, influenced by multiple factors, including multiple genes, environmental influences, and by developmental factors. A complex disease, or high level phenotype, may be considered as determined by a number of intermediate phenotypes, which in turn are determined by lower level phenotypes

Table 1 Intermediate phenotypes and possible candidate genes (low level phenotypes) for OSAHS

Intermediate phenotypes	Candidate genes
Obesity	**Leptin/leptin receptor**
	Pro-opiomelanocortin
	Insulin growth factor
	Glucokinase
	Adenosine deaminase
	Melanocortin-3 receptor
	Tumor necrosis factor α
	Glucose regulatory protein
	Agouti protein and protein related peptide
	β-3 Adrenergic receptor
	Orexins
Ventilatory control	RET-proto-oncogene, receptor tyrosine kinase
	Neurotrophic growth factors (BDNF, GDNF)
	Endothelin-1
	Endothelin-3
	Krox-20
	Retinoic acid receptor
	Leptin/leptin receptors
	Orexins (?)
Craniofacial dysmorphisms	Homeobox genes
	Growth hormone receptors
	Growth factor receptors
	Retinoic acid receptor
	Endothelin-1
	Collagen type I and II
	Tumor necrosis factor α
Sleep regulation	**Orexins**
	Leptin

Note: Items in bold are associated with more than one OSAHS intermediate phenotype.

(Table 1). Specific gene products may more directly influence lower level phenotypes than higher level phenotypes. Traits such as facial and head form, ventilatory chemosensitivity, load compensation, sympathetic nervous system activity, connective tissue laxity, muscle fatigability and central obesity are possible intermediate phenotypes for OSAHS. Examples of low level phenotypes, most of which are known to be influenced by specific genes, include hormone levels (e.g. insulin, leptin) or receptor subtypes (e.g. serotoninergic and retinoic acid receptors). Intermediate and low level phenotypes may be measured and defined more easily than a "complex" disease or syndrome, and, thus, could be employed as alternative phenotypes for studying the genetics of OSAHS.

Evidence for familial aggregation and inheritance

Despite the limitations of utilizing uni-dimensional phenotype definitions (i.e. AHI level), there has been accumulating evidence from clinical and epidemiological studies that support the importance of familial, and probably genetic, factors, in the expression

of OSAHS. The evidence for a genetic basis for OSAHS has been developed from the application of increasingly more quantitative methods to the study of this disorder. These have included descriptive reports of families with multiply affected members; studies of OSAHS prevalence among relatives of affected probands; quantification of the familial aggregation of OSAHS by comparing the prevalence of OSAHS among relatives of affected probands with that in control samples; and segregation analysis. Studies have included samples from the US, the UK and Israel. Information from these studies has been useful in establishing a likely role for inheritance apart from familial influences related to obesity. Preliminary results from segregation analysis have further defined the likely magnitude of genetic influences.

Family studies

Initial reports that address the familial basis of OSAHS included four studies of multigenerational families with multiple affected members [6–9]. OSAHS was reported in members of the same and different generations. It was found in children as well as adults, and in obese and non-obese family members. One report cited the cooccurrence of OSAHS, seizures and anosmia in affected family members as suggesting an inherited syndrome [8]; however, to our knowledge, this constellation has not been reported since. The report by El Bayadi *et al*. that included cephalometry and tests of ventilatory responses to chemical loading, demonstrated blunted ventilatory responses to progressive eucapneic hypoxia ventilatory challenges in all five of the affected subjects studied [9]. Thus, in this family, the underpinnings of OSHAS may have been associated with inherited abnormalities in the control of ventilation. Additionally, the importance of anatomic factors that influence upper airway size was suggested by the observation that the two most severely affected family members also had the longest soft palates and most inferiorly displaced hyoids. Together, these findings suggest that expression and severity of sleep apnea may result from interactions of physiological and anatomical abnormalities, each with a probable familial basis [9].

Community studies that address the familial clustering OSAHS symptoms

Four epidemiological studies (two of which were twin studies) have evaluated the familial aggregation of symptoms of OSAHS [10–13]. Kaprio *et al*., in their study of >4000 Finnish twins, found that the concordance for snoring was greater between monozygotic (MZ) twins than between dizygotic (DZ) twins, suggesting a role for inheritance [10]. Ferini-Strambi *et al*., studying 776 twin pairs, showed stronger associations between known risk factors and snoring in DZ twin pairs discordant for snoring than in MZ discordant twin pairs, which also was interpreted as evidence that genetic factors predispose to snoring [13]. Redline *et al*. estimated the degree of familial aggregation of several symptoms of OSAHS in families of probands with polysomnographically proven OSAHS and in control families [12]. Habitual snoring, excessive daytime sleepiness, and snorting, gasping, or apneas were reported two or four times more frequently among the first-degree relatives of patients with OSAHS than among control subjects. These findings were independent of familial similarities in body mass index (BMI), smoking and alcohol consumption, as well as of age and gender. Among 3308 men aged 54–74 years of age who participated in the Copenhagen

Male Study, a prospective cohort study, a significant relationship was demonstrated between family history of snoring and self-reported snoring [11]. Risk of snoring was increased approximately three-fold when at least one first-degree relative was reported to be a snorer, and increased four-fold when both parents were reportedly snorers. These relationships were independent of age, BMI, blood pressure levels and cardiovascular risk factors (including smoking, lipid levels and physical activity levels). Thus, symptoms associated with OSAHS aggregate significantly within families and are not fully explained by the familial clustering of other factors.

Familial clustering of AHI levels

Significant family clustering of apneic–hypopneic activity has been demonstrated in populations from Israel, the UK, northern California, and Cleveland, OH, USA [14–18]. In these studies, disease was defined based on various threshold values of the AHI, with and without other associated symptoms and signs. Defined in these ways, the prevalence of OSAHS among first-degree relatives of probands with OSAHS varied from 21% (in the Cleveland Family Study) [17] to as high as 84% (in the California sample) [18]. Likewise, among the studies which included control samples, the odds ratios, relating the likely existence of an individual with OSAHS in a family with affected relatives to the likelihood for someone without an affected relative, have varied from 2 to 46 [16–18]. The Cleveland study also assessed the familial aggregation of the AHI considered as a continuous variable and adjusted for age, BMI and gender. In this study, approximately equivalent parent–offspring and sib–sib correlations [17] were demonstrated for age, gender and BMI-adjusted AHI levels. Thus, the available data are consistent in demonstrating familial aggregation for OSAHS; however, the magnitude of increased risk attributable to family membership is not clear.

Differences in the estimates of relative risk could be due to several factors, including chance, differences in the populations studied and different characterizations of phenotype. Chance alone could explain some of the differences in the strength of the associations among studies (as evidenced by the nearly two-fold difference in prevalence among relatives studied by the same UK investigators 1 year apart [14, 16]), and/or by sampling biases, associated with differential participation rates of symptomatic relatives and controls. The populations targeted for each study varied in respect to age ranges, family structures and obesity levels. The only relatives studied by the Israeli and the UK investigators were the adult offspring of affected probands [14–16]. In contrast, the US studies included all available first-degree relatives with few age restrictions [17,18]. The analysis of data from across a range of ages requires consideration of the age dependency of OSAHS, and of potential differences in penetrance that may vary as a function of age as well as being influenced by age and gender interactions. In the UK studies, only probands with BMIs $<30\ kg/m^2$ were recruited [14,16]. The other studies enrolled patients with laboratory confirmed OSAHS, regardless of associated obesity. Estimates of genetic risk could be lower or higher among non-obese than obese individuals, according to the degree to which obesity directly or interactively influences the expression of OSAHS.

Phenotype definitions varied between and within studies. In the California study, when OSAHS was defined by an AHI >5 occurring with daytime tiredness or sleepiness and one other symptom, prevalence among first-degree relatives was 84% [18]. When also requiring the finding of a high, narrow hard palate (by physical examination) to

define OSAHS, prevalence dropped to 68% and the odds ratio fell from 46 to 11. In the Cleveland study, 21% of all first-degree relatives were characterized as "affected" with the use of an age-dependent AHI threshold level [17]. Changing the definition by requiring an AHI >15 and the occurrence of daytime sleepiness reduced the prevalence to 13% and increased the odds ratio from approximately 2 to 5. These considerations emphasize the importance of carefully considering how phenotype is defined. The Cleveland study also found an association between sudden unexpected death in infancy and OSAHS, suggesting that there are some families that may be predisposed to both syndromes [19].

Mode of inheritance

In the Cleveland Family Study, pedigree analysis demonstrated that OSAHS occurs as a familial disorder, affecting ≥ 2 members, in 114 of 175 families with an affected proband (65%), and as a "sporadic" disorder in 61 families (35%) (unpublished data). This suggests that familial OSAHS occurs more often than the "sporadic" form, although other explanations are possible. Examination of individual pedigrees demonstrates a number of families with affected members in multiple generations. The latter is consistent with (but not proof of) a Mendelian mode of inheritance; the operation of environmental factors common to families must also be considered.

Segregation analysis, which provides rigorous tests of the likelihood of the observed phenotypes based on specified genetic hypotheses, provides a more formal approach to identifying mode of inheritance. Such work is also useful for exploring alternative phenotype definitions: those definitions in which the highest heritability estimates are demonstrated may be the definitions that could provide the greatest evidence for linkage.

Work to date that has employed segregation analyses to assess the genetics of OSAHS is still preliminary. Segregation analyses of self-reported snoring in 584 pedigrees studied as part of the Tucson Epidemiologic Survey of Obstructive Airways Disease suggested a major gene effect; however, the evidence for this weakened after adjustments were made for obesity and gender [20]. Segregation analyses are underway using data from the Cleveland Family Study. Initial analyses, restricted to the Caucasian members of the cohort, suggest that the AHI is best explained by two underlying distributions, with a spousal correlation of 0, and parent–offspring and sib–sib correlations of 0.23 each [21]. The regressive Mendelian model that best explains the data is one in which two normal distributions of power-transformed AHI are due to the segregation of a dominant gene with a gene frequency of 0.046. This model was most consistent with dominant gene segregation that accounted for 12% of the variance in the sex, age and BMI-adjusted AHI level. These findings are being pursued with linkage studies, which are needed to identify specific genes that could account for these findings.

Racial and ethnic variations

Examination of racial and ethnic differences in OSAHS may shed light on underlying genetic mechanisms for disease. Although relatively little is known about OSAHS in non-Caucasian populations, emerging data suggest that certain races may be at

increased risk. Data from the Cleveland Family Study [22] and from a San Diego study of the elderly [23], demonstrated greater levels of AHI in African–Americans as compared to Caucasians. The San Diego study showed that elderly African–Americans were at an approximately two-fold increased risk for sleep apnea compared to elderly Caucasians, and also had more severe sleep apnea [23]. In the Cleveland family study, racial differences were most prominent in individuals <25 years of age (odds ratio = 1.88 in African–Americans vs Caucasians), and even higher among children <13 years (odds ratio >3.0) [22]. Racial differences were not accounted for by differences in BMI, alcohol exposure or tobacco use, suggesting that racial variations may be due to differences in upper airway anatomic and possibly physiological factors. Anatomic risk factors in African–Americans appeared to be related to increased upper airway soft tissue rather than bony features that reduce airway size [22]. Preliminary data from the Sleep Heart Health Study, a multi-center community based study of >6400 US adults, suggests that Native Americans and Hispanics may be at increased risk for sleep apnea, perhaps partly because of obesity in these groups (report in preparation).

Data from international studies also provide evidence for racial and ethnic differences in OSAHS. The extent to which racial variation relates to differences in obesity or body fat distributions are unclear. Using data from a referral clinic, Baldwin *et al*. have reported an excess of OSAHS and more severe OSAHS in Pacific Islanders and Maori living in New Zealand as compared to individuals of European descent [24]. These racial effects were attributed to group differences in neck size and body mass. A community based survey of almost 2300 residents of Singapore demonstrated ethnic differences in both snoring and a sleep apnea complex (defined by apneic symptoms, occurring with hypertension or a wide neck) [25]. In this sample, Chinese individuals had lower rates of snoring and evidence of sleep apnea as compared to Malays and Indians. After adjusting for obesity and age, the odds of sleep apnea were approximately 3 and 2 in Indians and Malays, respectively, as compared to Chinese. Further identification of the extent to which environmental or cultural factors versus genetic factors explain such differences could shed considerable light on underlying pathogenetic mechanisms for OSAHS.

Risk factors for OSAHS and their genetic bases

OSAHS may be considered a complex disease that is expressed after some threshold level of susceptibility is exceeded. Susceptibility relates to the predilection for repetitive upper airway collapse. In any given person, this is determined by anatomic and neuromuscular factors that influence upper airway size and/or function. The strongest risk factors for OSAHS are obesity and male gender [26]. Other risk factors are associated with hard and soft tissue features of the upper airway [27–29] and characteristics of ventilatory control [30–32]. Many of these factors vary with age, which also can be considered a risk factor. These risk factors are likely determined by genes, possibly modulated by environmental influences. The expression of many traits associated with OSAHS also may be influenced by developmental exposures, which may impact the development of craniofacial structure and central and peripheral nervous system functions. Obesity, craniofacial structure and ventilatory control, three areas that both appear to be substantially influenced by genetic factors and may influence the expression of OSAHS, are reviewed below.

Obesity and body fat distribution

Obesity appears to increase risk of OSAHS approximately 10–14-fold, with the most marked effects observed in middle-aged subjects [33–36]. Conversely, weight loss—even modest amounts—may reduce the severity of OSAHS [37]. Obesity may increase susceptibility to OSAHS through fat deposition in upper airway tissues, reducing nasopharyngeal caliber and/or from hypoventilation occurring in association with reduced chest wall compliance. It is also possible that the association between obesity and OSAHS may be partly based on pleiotropic effects, as may occur if the same gene or set of genes influences ponderosity and ventilatory control and/or craniofacial morphology.

The results of twin and family studies suggest that between 40% and 70% of the variance in measures of obesity (BMI, fat mass, skin fold thickness, etc.) within populations may be attributed to genetic factors [37–39]. Furthermore, segregation analyses suggest that a few genes may account for a large proportion of this variance [40]. Genetic factors that influence metabolic rate, thermogenesis, fat storage and eating behavior, and that are associated with abnormalities in autonomic, endocrinological and hypothalamic functions, are thought to contribute significantly to the development of obesity [39, 41–43]. Genetic factors also may be important in influencing regional body fat distribution and may account for as much as 25% of the intersubject variability in this trait [44]. This may be of particular relevance to the pathogenesis of OSAHS, in which upper body obesity may be a relatively greater risk factor than is total body fat mass. Even relatively non-obese individuals with OSAHS may have regional excess fat deposition, especially in the anterolateral upper airway [45].

A detailed review of the enormous amount of work that has been directed towards understanding the genetics of obesity can be found in [40]. Animal studies have suggested numerous potential candidate genes; however, only a few humans appear to have genetic mutations in homologous genes. Of great interest, however, are results from a genome wide scan conducted on 10 extended Mexican–American families, which established linkage between levels of serum leptin (an adipose derived circulating hormone) and areas on chromosomes 2 and 8 [46]. These regions respectively encompass genes encoding for pro-opiomelanocortin (POMC), which may be important in appetite regulation, and for β-3-adrenergic receptor (ADRB3), which may influence the regulation of energy expenditure. A more recent family study in Germany has additionally implicated mutations in the melanocortin-4 receptor gene (MC4-R) in extreme and moderate obesity [47].

Candidate genes for obesity are relevant for studies of the genetics of OSHAS both because of the prominence of obesity in the OSAHS phenotype, and because of the potential impact of these genes on the expression of other traits of potential relevance to OSAHS. For example, a number of candidate genes for obesity (e.g. leptin, adenosine deaminase and melanocortin-4 receptor) are expressed in a variety of tissues and brain sites important in the regulation of breathing [48,49]. There is growing evidence that leptin, specifically, may have pleiotropic effects. In addition to its role in appetite regulation and energy expenditure, mouse models suggest that leptin also influences lung growth [50] and respiratory control [51]. Knockout mouse models and studies after leptin replacement suggest that leptin deficiency causes depressed ventilatory responses to hypercapnia in both wakefulness and sleep [51]. Leptin administration also influences sleep architecture in rats [52]. If confirmed in humans, this could

provide important supporting data implicating the actions of a single gene on several aspects of the OSAHS phenotype (obesity, ventilatory control and sleep architecture).

Craniofacial morphology

Craniofacial morphology, affecting both bony and soft tissues, are thought to predispose to OSAHS by reducing the size of the upper airway [28]. Structural abnormalities that have been described in patients with OSAHS include reduction of the anterorposterior dimension of the cranial base [53], a reduced nasion–sella–basion angle [54], reduction of the size of the posterior and superior airway spaces [53], inferior displacement of the hyoid [29], elongation of the soft palate [55], macroglossia, adenoidotonsillar hypertrophy and increased vertical facial dimension, with a disproportionate increase in the lower facial height [53]. Retrognathia, micrognathia and type II malocclusions have been reported, although less consistently, among patients with OSAHS [56]. A brachycephalic head form (cephalic index >0.81) is often found in association with reduced upper airway dimensions. In Caucasians, this head form is associated with an increased risk of OSAHS, and also identifies families at risk for both OSAHS and sudden unexpected death in infancy [19]. In people of African descent, this head form is uncommon, and does not appear to increase risk of OSAHS [22]. Whether brachycephaly is a risk factor in people of Asian descent, in whom it occurs frequently, is not known. OSAHS is common in individuals with Downs' syndrome, which is commonly associated with a number of craniofacial dysmorphisms.

A genetic basis for craniofacial morphology is suggested by twin and family studies. Nance *et al*. have applied multivariate analytic techniques to the analysis of cephalometric data from 24 MZ and 21 DZ twin pairs of the same gender, aged 10–17 years [57]. A high heritability estimate (>0.59) was found for all eight cephalometric parameters examined, including several measures that have been reported to identify patients with OSAHS (the distance between basion and nasion; the overall length of the cranial base, and the nasion–sella–basion angle). Further statistical analysis indicated that at least four significant independent measures, apparently inherited, contributed to the overall variation in these measurements: the first influenced primarily two horizontal mandibular measurements; the second, the horizontal cranial base measurements and the mandibular gonial angle; the third, the nasion–sella–basion angle; and the fourth, the posterior cranial base and the mandibular ramus. All of these factors may influence the relative patency of nasopharynx. Osborne and De George, analysing data from approximately 60 MZ and 40 DZ twins, estimated the heritability of a number of measures of craniofacial structure. The heritability of one of these, the cephalic index, was extremely high (0.90 in males, 0.70 in females) [58]. Heredity appeared to account for 40% of the variability of dental and facial characteristics associated with malocclusions [59]. In humans, micrognathia can be found in a myriad of chromosomal deletion syndromes, suggesting that normal craniofacial growth may be influenced by a number of genes, perhaps with specific dosage requirements.

Inherited abnormalities of craniofacial structure appear to explain at least a portion of the familial clustering of OSAHS. In a study from the UK, relatives of OSAHS probands were shown to have decreased total pharyngeal volumes and glottic cross-sectional areas, retropositioned maxillae and mandibles, and longer soft palates compared with relatives of controls [16]. In a northern Californian sample, relatives of patients with OSAHS were shown by cephalometry to have a more retropositioned

mandible and smaller posterior superior airway space as compared to normative data [18]. In the Cleveland Family Study, both hard tissue (e.g. head form, intermaxillary length) and soft tissue factors (e.g. soft palate length, tongue volume) predicted the AHI level in Caucasian families. In Black families, soft tissue factors also predicted AHI levels, but hard tissue anatomic features appeared to be only weakly associated with OSAHS [22]. These data support the importance of structural features in increasing susceptibility to OSAHS, but also suggest that the anatomic underpinnings, and thus the genetics, for OSAHS may differ among racial groups.

Mouse models suggest that a number of different genes may influence craniofacial development. Craniofacial abnormalities, including retrognathia and micrognathia, have been described in mice deficient in growth and differentiating factor transforming growth factor-β2 [60] and in mice with collagen gene mutations (types II and XI). Defects also have been described in mice deficient for the retinoic acid receptor-αγ [61] and endothelin-1 [62]. Further understanding of homeobox genes and genes controlling growth factors may contribute to our clarifying the origins of craniofacial dysmorphisms found in OSAHS.

Ventilatory control patterns

Airway patency is influenced dynamically by an array of complex processes associated with the control of both chest wall and upper airway neuromuscular function. Potentially inherited abnormalities of ventilatory control may predispose to obstructive or central sleep apnea or both by influencing ventilation during sleep and increasing the propensity to upper airway collapse. This might occur by preferential reduction in the level of activation of upper airway muscles as compared to chest wall muscles [2]. Altered ventilatory drive also may precipitate apnea by promoting ventilatory control instability and, subsequently, periodic breathing [63]. In this regard, ventilatory control instability could result from either blunted or augmented chemosensitivity [63,64]. Abnormalities in respiratory responsiveness to chemical or mechanical stimulation may prolong the duration of apneas by impairment of the arousal response to the obstructive episode. This notion is supported by the demonstration that the degree of oxygen desaturation is the greatest and the duration of apneas the longest in subjects with OSAHS in whom ventilation in response to hypoxia during wakefulness is the most blunted [30].

An inherited basis for ventilatory responsiveness to hypoxemia or hypercapnia has been suggested by the findings from several human studies. Abnormalities in ventilatory responsiveness to hypoxia and/or hypercapnia have been described in the first-degree relatives of probands with various pulmonary diseases or syndromes, including unexplained respiratory failure [65,66], chronic obstructive pulmonary disease [67–69] and asthma [70]. A genetic basis for the chemoresponse to blood oxygen saturation is suggested by several twin studies that have demonstrated similarities in ventilatory responses to hypoxia or hyperoxia to be greater in MZ than in DZ twins [71–73]. Heritability estimates for chemoresponsivity to oxygen saturation levels vary between approximately 30% and 75%, suggesting a substantial contribution of inheritance to this trait [73,74]. Evidence for a role of genetics in the ventilatory response to hypercapnia in humans is less consistent, however [75–77].

The potential importance of inherited impairments of ventilatory control in influencing susceptibility to OSAHS has been suggested by several studies of carefully

characterized families. In one family with nine affected members, we demonstrated depressed hypoxic responses in all five affected members in whom this was assessed [9]. Later studies in 22 subjects from 13 families in which multiple members had OSAHS, and in a control sample derived from nine families with no member with OSAHS included measurements of respiratory responses to hypoxia, hypercapnia and ventilatory loading [78]. Members of OSAHS families demonstrated significantly reduced ventilatory responses to progressive eucapnic hypoxia measured during wakefulness as compared to members of control families. Additionally, impairment in load compensation was suggested by the finding of a significantly greater increase in ventilatory impedance with inspiratory resistive loading in OSAHS family members as compared to control subjects. This finding is consistent with the observations made by Lavie *et al*. who described increased apneic activity following nasal occlusion and impaired load compensation during sleep in relatives of OSAHS patients as compared to controls [78,79]. These data suggest that the familial aggregation of OSAHS may in some instances be based on inherited abnormalities in ventilatory control, perhaps related to chemoregulation and/or load compensation. The upper airway of genetically susceptible individuals appears vulnerable to excess collapsibility during conditions of mild inspiratory loading. This may occur especially during sleep as the balance between upper airway and chest wall activation changes or intrathoracic airway pressure during inspiration becomes more negative.

Some clues regarding the underlying genetics of ventilatory control abnormalities in OSAHS may be gleaned from studies of children with congenital central hypoventilation (CCH) syndromes. Although the phenotype of these latter syndromes may be much more dramatic and specific than that of OSAHS, it is reasonable to consider the possibility that these disorders share common underlying genetic defects, possibly present in different "doses" or differentially modified by other factors. There are numerous case reports of children with frequent apneas and daytime hypoventilation that appear attributable to severe chemoregulatory dysfunction, manifest as profound blunting of the hypercapnic and hypoxic ventilatory responses [80]. Developmental abnormalities of the brainstem or cerebral cortex have been found in some cases. In the absence of secondary causes, such cases are labeled as "idiopathic congenital central hypoventilation" ("Ondine's curse"). Familiality in CCH has been described [81–83], and complex segregation analyses suggest that the disorder can be explained by either multifactoral threshold or major locus models [84]. Interestingly, Hirschsprungs disease, a congenital disorder characterized by intestinal dysmotility and absence of myenteric and submucosal ganglia in the distal bowel [85], may occur in as many as 50% of cases of CCH [86]. Mutations of both the RET proto-oncogene, encoding a receptor tyrosine kinase thought to be involved in neural crest migration and proliferation, and the RET ligand, glial cell line-derived neurotrophic factor (GDNF) [87], have been described in children with Hirschsprungs disease, and in CCH occurring in association with Hirschsprungs disease [88–91]. These associations suggest that CCH syndromes sometimes may be caused by abnormalities in migration of neural crest cells to central respiratory control centers [88]. Other genes involved in the endothelin signaling pathway (endothelin B receptor gene, EDNRB and endothelin 3 gene, EDN3) have been implicated in Hirschsprungs disease also and could be considered candidate genes for CCH syndromes and sleep apnea [92].

Knockout and transgenic mouse models have been used to identify genes potentially important in the control of ventilation. Heterozygous and homozygous RET knockout mice, who survived only briefly, demonstrated reductions in hypercapnic ventilatory

responses [93]. These studies provide important support to the observations described above in humans regarding mutations in this gene and CCH. Endothelin-1 (ET-1), a potent vasoactive peptide, may also participate in control of ventilation. In a knockout mouse model, absence of ET-1 results in respiratory failure, ventilatory control abnormalities, craniofacial abnormalities and hypertension, characteristics remarkably similar to traits found in OSAHS [62]. Mutant mice deficient in ET-1 have impaired ventilatory responses to both hypoxia and hypercapnia [94]. The findings of reductions in both responses suggest impairment of central control mechanisms. A European group has identified the zinc finger protein Krox-20, which affects the development of the hindbrain. When it is deleted by homologous recombination, mice demonstrate slow respiratory frequencies and long apneas [95]. Erickson *et al.* have shown in a knockout mouse model that loss of brain derived neurotrophic factor (BDNF) results in reduced survival of neurons in the nodose-petrosal ganglion [96]. Homozygous mice demonstrated irregular and depressed ventilation, including spontaneous apneas, and abnormalities in chemoregulation specifically related to hyperoxia but not to hypercapnia. Heterozygous mice demonstrated ventilatory responses intermediate to those of the wild-type and homozygous mice, suggesting that non-lethal alterations in the genetic control of neural growth factors may contribute to phenotypic variations in ventilatory traits [96]. Together these data underscore the complexity of the respiratory control system, and indicate that a number of genes, important in different native regulatory functions, may influence ventilatory phenotypes.

Using a different approach—a mouse intercross strategy—Tankersley and associates have studied the inheritance of respiratory patterns, assessed both at rest and in response to chemo-challenges [97–100]. They observed significant inter-strain differences in respiratory pattern (frequency, tidal volume and inspiratory timing) [98]. The pattern of inheritance was most consistent with the operation of a small number of genes. Linkage was reported between phenotypic differences in inspiratory timing and two putative quantitative trait loci on mouse chromosome 3 [99]. They suggested that candidate genes include a family of genes that encode neuroreceptors (e.g. glycine receptor, glutamate receptor) and genes that influence the postnatal development of the lung (e.g. basic fibroblast growth factor, bFGF). Further work by this group has examined breathing frequency during hypoxia. Preliminary findings suggest that a two-gene model, involving genes that are different from those that determine baseline frequency, best explains this phenotype [100]. Strohl *et al*. reported measurements of ventilation and metabolism in four inbred strains of rats chosen for a wide variation in body weight and/or blood pressure regulation [101,102]. Significant differences in the pattern of breathing persisted when animals were exposed to 100% oxygen, an observation that suggests genetic differences in the central regulation of breathing pattern.

Genes that influence sleep and circadian rhythm

Given the impact of state (sleep–wake) on respiratory motorneuronal activation, a comprehensive understanding of the susceptibility of upper airway muscles to collapsibility during sleep may require delineation of the genetics of sleep–wake control. Some of the most exciting work on sleep–wake control has come from recent studies of narcolepsy. Experimental studies in both dogs and mice have implicated deficiencies in the orexins (hypocretins; two polypeptides that are ligands for two G protein-coupled receptors in the brain) in causing the phenotypic abnormalities in

sleep regulation characteristic of narcolepsy (cataplexy, REM-onset sleep and hypersomnolence) [103–105]. Neurons containing orexins are located exclusively in the lateral hypothalamus, an area thought important both for sleep regulation and circadian rhythm. In mice, orexin neurons have been demonstrated to have widespread projections to areas in the ascending cortical activating system, including the tuberomamillary nucleus, locus coeruleus, the dorsal and median raphe and pedunculopontine nuclei [103]. The pedunculopontine nuclei are thought to be especially critical to the control of REM sleep. Projections from these nuclei to the pontine reticular formation, in turn, may be involved in REM atonia. It seems plausible to speculate that abnormalities in orexin genes, or genes coding for their receptors, could be relevant to studies of OSAHS because of the potential impact of these neuropeptides on arousal and muscle tone, both of which influence the behavior of respiratory systems, and/or because of the close proximity of these neurons to central respiratory control centers, with potential interactions between arousal and respiratory centers.

In addition to considering the impact of genetic abnormalities on processes that regulate sleep–wake state, it is also important to consider how respiratory motorneuronal control may be influenced by genetic processes that determine circadian clocks, known to drive important metabolic and behavioral rhythms. Drosophila and mouse models have identified a number of genes that influence periodicity and persistence of circadian rhythms [106,107]. The impact of genetically determined disorders of circadian rhythm could be of relevance to research in OSAHS, although this interaction has not been subject to much study.

Candidate genes and biochemical markers

Numerous genes have been identified in rodents that influence the expression or regulation of proteins or receptors that may be considered low level phenotypes for OSAHS (Table 1). Genes that influence the development of the hindbrain, cervical spinal cord and nodose petrosal ganglionic may be anticipated to influence the control of breathing and possibly craniofacial structure. Genes that influence obesity and body fat distribution and the sleep–wake state also may be important in OSAHS. These generally include the family of homeobox genes (Hox), zinc finger genes [95], genes involved in neural crest differentiation and development, intermediate [61,62] and neuronal growth factors (GDNF and BDNF) [96], members of the transforming growth factor-β family [60], orexins and their respective receptors. Of interest, is that several genes and gene systems may be influential in more than one intermediate OSAHS phenotype (see bold items in Table 1). Other candidate genes may influence several traits relevant to OSAHS. Abnormalities in fibrillin, as found in Marfan syndrome, may contribute to both craniofacial dysmorphism and upper airway connective tissue laxity [108]. Other loci of interest may be elsewhere on chromosome 15, mutations of which may result in a number of somatic abnormalities (e.g. Prader–Willi syndrome) as well as OSAHS. A preliminary study from China, reporting an association between a polymorphism between the angiotensin converting enzyme gene and severity of sleep apnea, suggests the potential importance of this gene, or genes near this gene, in moderating expression of OSAHS [109].

Genetic studies would be greatly enhanced by identification of good biochemical markers for traits associated with low or intermediate phenotypes for OSAHS. The distribution of various biochemical markers in samples of OSAHS patients has been

examined to understand how end-organ effects of OSAHS, such as sleepiness or hypertension, may be mediated. A number of studies have examined catecholamine, sex and growth hormone levels in OSAHS. However, data from these studies have not been used for phenotypic characterization of groups of subjects with OSAHS. Elevations in levels of circulating endothelin-1 [110], a peptide with vasoconstrictor effects, and in the inflammatory cytokine, tumor necrosis factor, have been demonstrated in OSAHS subjects as compared to control subjects [111,112]. These elevations could be secondary effects resulting from apnea-mediated hypoxia or adrenergic stimulation, or from the obesity of the OSAHS subjects, rather than from primarily genetically aberrant physiological systems. Similarly, plasma fibrinogen concentration and whole blood viscosity have been reported to be higher in the morning than afternoon in a small number of untreated OSAHS patients, with no such diurnal change in treated OSAHS patients [113]. Variations in levels of heat shock proteins, proteins thought to respond to stresses such as hypoxia, have been examined in small numbers of OSAHS patients, with results that were somewhat equivocal [114]. Further exploration of the variation in markers of stress, particularly focused at elucidating whether any observed biochemical perturbation is a primary or secondary phenomenon, is needed. Future investigations should also consider studying markers associated with derangements in neurotransmitters involved with central ventilatory control or with sleep homeostasis as means for better describing the OSAHS phenotype.

There has been minimal investigation of genetic markers in OSAHS. An approximately two-fold increase in the HLA-A2 antigen was demonstrated in a sample of Japanese subjects with OSAHS as compared to age-matched controls [115]. HLA-A2 positive subjects with OSAHS were more obese than OSA patients negative for this antigen, suggesting a relationship between this genetic marker and obesity. However, other phenotypic differences were not examined. HLA-DR2, which is strongly associated with narcolepsy, has not been found to be associated with OSAHS [116]. A higher prevalence of the Lewis blood group phenotype Le(a+b−) was found in those who reported very disruptive snoring as compared to other snorers who comprised a subsample of Danish subjects with habitual snoring [11]. The implication of this observation, which has not been confirmed, is not clear.

Summary

Despite the challenges in studying an inherently complex trait, there is growing evidence from clinical and epidemiological studies that genetic factors importantly influence the expression of OSAHS. The overall magnitude of effect that may be attributable to genetic factors and whether the disorder is due to genes of large effect requires further definition. Preliminary segregation analyses from the Cleveland Family Study are consistent with the operation of a few genes of moderate effect, that may explain about 12% of the variance in the AHI in Caucasians [21]. Although additional efficiency may be anticipated by describing samples with relatively homogeneous characteristics, this is currently difficult to do in OSAHS because disease often appears to be expressed because of the interaction among a number of risk factors. The identification of biochemical "markers" to improve disease classification would certainly help elucidate underlying pathogenic processes, as well as facilitate genetic studies. In parallel with human studies has been the development of rodent models to identify genes that influence the expression of traits that may underlie OSAHS. The

successful iteration of information gleaned from human and animal work should lead to the mapping of genes for traits that substantially influence the expression of OSAHS.

Practice Points

1. Many of the established risk factors (e.g. obesity, body fat distribution, craniofacial morphology) for the OSAHS have a known or suspected genetic basis.
2. OSAHS has been demonstrated to aggregate significantly within families. The risk of sleep apnea may be 2–4-fold greater in relatives of patients with sleep apnea as compared to controls. Nearly 40% of the variance in the AHI may be explained by familial factors.
3. Estimates of heritability have not been appreciably influenced by adjustments for BMI. This suggests that there may be physiological and/or unmeasured anatomic factors that both are inherited and that predispose to OSAHS.

Research Agenda

1. Additional work is needed to identify biochemical markers for characterizing underlying physiological mechanisms for OSAHS, distinguishing parameters that reflect the consequences of the disease from those that indicate differences in inherent susceptibility.
2. Further investigations of the pathophysiology of OSAHS should be aimed at identifying intermediate phenotypes that could be readily measured in large population samples.
3. Additional studies of rodent models of disease should be used to inform human studies.
4. Further studies, comparing populations with distinct ethnic and racial compositions, should be conducted to identify the role of genetic and environmental factors that influence disease susceptibility.
5. Further studies of the interactions between genes that influence sleep–wake state and those that influence respiratory control are needed.

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