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ORIGINAL INVESTIGATION

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Altitude is a phenotypic modifier in hereditary paraganglioma type 1: evidence for an oxygen-sensing defect

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Abstract Hereditary paraganglioma type 1 (PGL1) is characterized by slow-growing and vascularized tumors that often develop in the carotid body (CB) and is caused by mutations in the gene for succinate dehydrogenase D (*SDHD*) of mitochondrial complex II. The mechanisms of tumorigenesis and the factors affecting penetrance and expressivity are unknown. Because chronic hypoxic stimulation at high altitudes causes sporadic CB paragangliomas, it has been hypothesized that the *SDHD* gene product may be involved in oxygen sensing. On this background, we examined genotype-phenotype-environment relationships and tested whether higher altitudes adversely affect the phenotype in PGL1. An analysis of 58 subjects from 23 families revealed that nonsense/splicing mutation carriers developed symptoms 8.5 years earlier than missense mutation carriers (P<0.012). We also found that subjects who were diagnosed with single tumors at their first clinical evaluation lived at lower average altitudes and were exposed to lower altitude-years than those with multiple tumors (P<0.012). Pheochromocytomas developed in six subjects (approximately 10%), five of whom had nonsense mutations (P=0.052). Subjects with pheochromocytomas also lived at higher average altitudes and were exposed to higher altitude-years than those without them (P=0.026). To test whether altitude is also associated with the more frequent detection of germ-line founder mutations among sporadic cases in The Netherlands than in the USA (P= 0.00033), we calculated population-weighted elevations of the two countries. We found that the population-weighted elevations were approximately 260 m for the US and 2 m for the central-western Netherlands (P~0), where three Dutch founder mutations were discovered. This finding suggests that low altitudes in The Netherlands reduce penetrance and relax the natural selection on *SDHD* mutations. Collectively, these data suggest that higher altitudes and nonsense/splicing mutations are associated with phenotypic severity in PGL1 and support the hypothesis that *SDHD* mutations impair oxygen sensing.

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Introduction

Hereditary paraganglioma (PGL) is characterized by the development of highly vascularized, slow-growing tumors that often develop in the head and neck region. PGL tumors derive from paraganglia, a diffuse neuroectodermal system distributed throughout the body. Paraganglia play important roles in organismic homeostasis against hypoxia, bleeding, cold, and hypoglycemia. The carotid body (CB), a highly specialized paraganglion located at the bifurcation of the common carotid artery, senses acute hypoxia and stimulates the cardiopulmonary system (Gonzalez et al. 1994). The CB in the head and neck region and the adrenal medulla in the abdomen are the two most common PGL tumor locations (Baysal 2002).

Mutations in the genes for succinate dehydrogenase B (*SDHB*), C (*SDHC*), and D (*SDHD*), which encode three of the four subunits of mitochondrial complex II (MTCII; Lancaster 2002), have been linked to the pathogenesis of

PGL. *SDHD* was the first gene to be identified with PGL1 at 11q23 by positional cloning (Baysal et al. 2000). The transmission of *SDHD* mutations displays a parent-of-origin effect. The disease phenotype occurs in an age-dependent autosomal dominant fashion after paternal transmission, whereas after maternal transmission, no disease phenotype is seen, suggesting the operation of genomic imprinting at the *PGL1* locus (van der Mey et al. 1989). The genomic imprinting rule in disease transmission has not been violated in any *PGL1*-linked pedigree (Heutink et al. 1992; Baysal et al. 1997, 1999), although the molecular basis of imprinting is as yet unknown: *SDHD* shows biallelic expression in several tested tissues (Baysal et al. 2000). The germ-line loss-of-function mutations in the paternal *SDHD* allele followed by somatic loss of heterozygosity (LOH) in the maternal allele suggests a tumor suppressor role for MTCII in paraganglionic cells (Baysal et al. 2000; Taschner et al. 2001). Mutations in *SDHC* (*PGL3*) and *SDHB* (*PGL4*) have subsequently been discovered in other paraganglioma families by direct candidate gene analyses (Niemann and Muller 2000; Astuti et al. 2001b). A joint analysis of the three *PGL* susceptibility genes in subjects with head and neck paragangliomas (HNPs) indicates that *SDHD* is the most commonly mutated gene (Baysal et al. 2002). A locus at 11q13 (*PGL2*) mapped in an extended Dutch pedigree could not be confirmed in independent pedigrees (Mariman et al. 1993). Mutations in *SDHB* and *SDHD* are also frequently detected in families and individuals who primarily present with pheochromocytomas (PHEOs; Astuti et al. 2001a; Gimm et al. 2000; Neumann et al. 2002). Whether there is a genotype-phenotype relationship in any of the *PGL* loci is unknown.

The population genetics of PGL1 shows peculiar characteristics. A large number of PGL1 families have been reported from The Netherlands. Following the initial linkage mapping to 11q23 in a large Dutch pedigree (Heutink et al. 1992), ten more families who share an ancestral haplotype and who lived within a 40-km radius of the city of Leiden have been described (van Schothorst et al. 1998). After the discovery of *PGL1* as the *SDHD* gene, two *SDHD* founder mutations, D92Y and L139P, were identified in a total of 50 familial and nonfamilial Dutch paragangliomas in one study from Leiden (Taschner et al. 2001). More recently, an L95P mutation has also been detected in nine Dutch subjects from the city of Rotterdam (Dannenberg et al. 2002) and one family from Nijmegen (Cremers et al. 2002), suggesting the presence of a third Dutch founder mutation. Together, these three studies describe 83 "unrelated" PGL1 families and subjects attributable to three founder mutations in the central and western parts of The Netherlands, in stark contrast to the 25 distinct *SDHD* mutations (reviewed in Baysal 2002) reported thus far among 43 independent familial and nonfamilial cases from the USA (Baysal et al. 2000, 2002; Milunsky et al. 2001), Australia (Badenhop et al., 2001), England (Astuti et al. 2001a), Germany/Poland (Neumann et al. 2002), France (Gimenez-Roqueplo et al. 2001), Spain (Cascon et al. 2002), and Belgium (Renard et al. 2002). The only PGL1 founder effect suggested outside of The Netherlands is P81L, detected in several US families (Baysal et al. 1999). However, P81L is evidently a recurrent mutation (Baysal et al. 2002), and so it is unclear what portion of these US families represents the descendants of a single founder or is a consequence of *de novo* mutations. The clustering of three of the four *SDHD* founder mutations in the central-western Netherlands is intriguing.

Furthermore, a high proportion of nonfamilial (isolated case) paragangliomas in The Netherlands carries a founder mutation. Thirty-three of 93 nonfamilial subjects (~35%) with HNPs had one of the three Dutch founder mutations (Dannenberg et al. 2002; Taschner et al. 2001). However, unsuspected *SDHD* germ-line mutations were uncovered only in two of 37 nonfamilial subjects (~5%) with HNPs in a US sample (Baysal et al. 2002), revealing a highly significant difference in the number of nonfamilial paraganglioma cases caused by occult germ-line *SDHD* mutations between the two populations (a comparison of mutation positive and negative nonfamilial cases by Fisher's exact test reveals a two-sided *P*-value of 0.00033). The reasons for this striking difference are unknown.

The increased incidence of paragangliomas in humans and other mammals living at very high altitudes (4,000 m above sea level) in the Andean mountains (Arias-Stella and Valcarcel 1976) and the subsequent confirmation of hypoxia as a proliferative factor for CB chief cells (reviewed in Baysal 2001) establish chronic hypoxia as a risk factor for sporadic paragangliomas. On the basis of these observations and the phenotypic similarity between sporadic paragangliomas and PGL1, it has been hypothesized that mutations in *SDHD* disrupts oxygen sensing in the CB and leads to tumor formation, thus effectively mimicking the pathogenesis of sporadic tumors at high altitudes (Baysal et al. 2000). In accord with this hypothesis, a PHEO tumor associated with an *SDHD* mutation was shown to express hypoxia-inducible factors at higher concentrations than those found in three sporadic counterparts (Gimenez-Roqueplo et al. 2001). Nevertheless, paraganglionic tissues are known to be hypoxia-responsive (Gonzalez et al. 1994), but whether oxygen sensing is defective in PGL1 is unknown. A testable prediction of this hypothesis is that atmospheric oxygen pressure, which is primarily determined by geographical elevation, would significantly modify genotype-phenotype correlation in *PGL1* such that higher altitudes would be associated with a more severe phenotype.

To investigate genotype-phenotype-altitude relationship on *PGL1* disease expression, we determined phenotypic characteristics and residential altitudes of 58 affected or at-risk carrier subjects ascertained from 23 unrelated families with established *SDHD* mutations. To explore further whether altitude plays a role in the frequent occurrence of Dutch founder mutations among nonfamilial cases in The Netherlands, we performed a comparative analysis of population-weighted altitudes of the central and western parts of The Netherlands, where founder effects have been detected, *vis-à-vis* the USA. Altogether, these findings indicate that the phenotypic expression and the population genetics of *PGL1* are influenced by alti-

tude and provide evidence for gene-environment interaction in tumor development.

Materials and methods

Subjects, families, and mutation analysis

The subjects in this study belong to the PGL families recruited through the University of Pittsburgh Paraganglioma Program. The recruitment of the subjects was approved by the IRB review committee of the University. A total of 58 affected subjects from 23 families were analyzed. Lifetime analyses were performed on 57 subjects, lifetime residential data being absent for one subject. All *SDHD* mutation carrier subjects whose residential and clinical information could be obtained were included in the analysis. Twenty-one families were recruited from the US, one was from Canada, and one was from Turkey. Mutation analysis was performed by direct sequencing on an ABI 377 automated sequencer with previously described primers and conditions (Baysal et al. 2000).

Data collection and analysis

Subjects

Phenotypic data were collected from multiple sources including physician and genetic counseling reports, clinical notes, pathology reports, telephone interviews, and mail surveys. Regardless of clinical status, surveys were sent to all members of PGL families who had *SDHD* mutations, and who had previously given consent for the study, to ensure confidentiality and to protect those who did not know their risk status. Only data from affected and carrier individuals were used in the study. The phenotype data collected through the surveys included simple measurements of phenotypic severity, such as tumor number, distribution, malignancy, age at symptom onset, and age at clinical diagnosis. Data concerning the locations where the participants had lived for more than 6 months were also collected. To corroborate the self-reported clinical information through independent sources, all participating affected and at-risk-carrier subjects were asked for their permission for us to open their medical records or to contact their health providers. Consequently, a subset of data provided by the subjects could be independently confirmed for 17 subjects through their medical records and for 20 subjects through communications with their physicians and genetic counselors. The confirmation of the clinical data in a subset of the subjects supported the reliability of the simple information gathered through the surveys and telephone interviews, although we cannot entirely rule out various sources of uncertainty inherent in medical history collection, such as recall bias. Review of the available medical records indicated that diverse radiological methods, including arteriography, computer tumography scan, and magnetic resonance imaging (alone or in combination), were used to determine the number and distribution of tumors. Although differential sensitivities of the imaging methods or the aggressiveness of the clinical workup may have influenced the number of detected tumors, we assumed that symptomatic and clinically significant tumors were more likely to be detected regardless of the specific radiological method used. The higher frequency of paragangliomas in autopsy series in the general population suggests that most asymptomatic paragangliomas are indeed clinically insignificant (Baysal 2002). In our study, to improve the reliability of the ascertainment of subjects with multiple tumors at the time of first diagnosis, individuals who had single tumors at the first clinical examination, and who were diagnosed with additional tumors within less than 2 years were considered to have multiple tumors at the time of initial diagnosis. All, except for two subjects with multiple tumors, were diagnosed in US hospitals.

Residential altitudes

The altitudes of the locations where the subjects lived were obtained from the United States Geological Survey (USGS) national mapping information databases available at the Department of Interior and at Topozone.com. A global gazetteer (available at http://www.lib.ohio-state.edu/refweb/resources/gazette.htm) was used to find the altitudes of international locations. Altitudes of three locations in the US, which were not available in the USGS database, were determined by telephoning the city hall or airport of the locations. It is possible that the exact altitudes of the subjects living in a given location may be different from those indicated in these databases. However, we assumed that such deviations should be random in the final analyses of a total of 153 and 168 subject-location points reported before the age at clinical diagnosis and during the lifetime of the subjects, respectively. Two altitude-exposure variables were derived for each subject: $h(t)$, which is the average altitude at which a subject lived up to a time point of interest, such as the age at symptom onset (ASO), age at clinical diagnosis (ACD), or current age (CA), and $h(t)\times t$, which is the total altitude-years of a subject up to a time point of interest, akin to a measure of cumulative lifetime risk during the life of each subject, where h is altitude, and t is time. Average altitudes were calculated by converting them first into barometric pressures, which directly determine atmospheric oxygen pressures. Converting altitudes into barometric pressure also allows handling altitudes, such as sea level (zero) or even below sea level. It should be noted, however, that using altitudes directly, without converting them into barometric pressures, yielded almost identical average altitudes (data not shown).

To convert the altitude of each geographic location to barometric pressure, we used the Model Atmosphere equation: $PB = \exp^{[6.63268 - 0.1112h - 0.00149(h\times h)]}$, where PB is barometric pressure in Torr, h is altitude in kilometers, and *exp* refers to the natural logarithm exponential function (West 1996). Although latitude and season can also locally modify barometric pressures, this formula provides a highly reliable estimate especially at altitudes below 2 km, at which all subjects in this study resided. Total barometric pressure, $BP(t)\text{-}_{total}$, to which a subject was exposed until t, a time point of interest in a subject's life, was calculated by summing the barometric exposures times, viz., the years spent in each location, i.e., sum(Torr×year). The $BP(t)\text{-}_{total}$ was then divided by t, age (in years) at the time point of interest in a subject's life, to find the average barometric pressure, $BP(t)$, exposed. $BP(t)$ was then converted back to average altitude, $h(t)$, by solving the quadratic Model Atmosphere equation (see above) for h. The value of $h(t)$ corresponds to an altitude that would deliver an equal amount of barometric pressure if the individual had lived in the same place until t, the time point of interest in a subject's life. The other variable, the total altitude-year, $h(t)\times t$, was derived simply by multiplying the average altitude, $h(t)$, by the time point of interest in a subject's life (t in years). Because this parameter includes both average altitude, $h(t)$, and the time, (t), spent at that altitude, it reflects individual differences in total barometric exposures within a sample more accurately than $h(t)$ alone.

Comparison of population-weighted altitudes of the US and The Netherlands

Population data were extracted from the Gridded Population of the World version 2 (GPW2) data set available through CIESIN at Columbia University (http://sedac.ciesin.columbia.edu/plue/gpw/index.html, accessible through www.ciesin.org). For the present calculations, we used a matrix containing 153,815 rows, one for each lowest-level administrative unit or subunit for which the population was known or could be estimated for 1990, and four columns. Columns 1 and 2 gave the longitude (x-coordinate) and latitude (y-coordinate) in signed decimal degrees, with Greenwich as zero longitude and the equator as zero latitude, of the centroid of each administrative unit or subunit. Column 3 gave the estimated 1990 population. The total population in column 3 was 5.18 billion. Dr. Christopher Small, Lamont Doherty Earth Observatory of Colum-

bia University, provided column 4, which gave the elevation (in meters above mean sea level) of each centroid (for methods and elevation data sources, see Cohen and Small 1998).

We approximated the USA and the central western region of The Netherlands by rectangular boxes of longitude and latitude. Each box contained all data units for which the centroid fell on or within the boundaries. Thus, a portion of the area and population of some of the data units located near a boundary may have fallen outside of the box. As the administrative units or subunits were very small compared with the size of each box, the effect of data units overflowing the boundaries of the box was probably negligible. No greater precision in reporting these estimates is justified in light of the uncertainty of the underlying data on population, elevation, and the boundaries of the relevant regions.

For each box, the population-weighted mean elevation (*mean_elev*) was computed as the sum (over all administrative units in the box) of the population (*ppn*) multiplied by the elevation of each unit (*elev*), the sum being divided by the total population in the box. Symbolically, $mean\_elev = \text{sum}(ppn \times elev)/\text{sum}(ppn)$. The population-weighted standard deviation of elevation (*sd_elev*) was computed as the square root of the difference between the mean squared elevation and the squared mean elevation. Symbolically, $sd\_elev = (\text{sum}(ppn \times elev^2)/\text{sum}(ppn) - mean\_elev^2)^{1/2}$. Here, "sum" means a summation over all data units with a centroid falling in or on the boundaries of the box.

Statistical analyses

Because of the relatively small sample sizes of the PGL1 patients and the known non-normal distribution of population by altitude (Cohen and Small 1998), we followed a conservative approach and used nonparametric methods for statistical testing: Fisher's Exact test for discrete counts, and Mann-Whitney U tests for continuous variables such as altitude. We also calculated the standard error (SE) of the mean for the continuous variables. The nonparametric Mann-Whitney U test, used in the comparison of altitude and altitude-year differences, depends on ranking and is thus less sensitive than, for example, a *t*-test, to the extreme values.

Table 1 Kindreds and genotypes of 58 subjects. Pedigrees PGL020 and PGL033 originated from Turkey and Canada, respectively

No.	Pedigree ID	Mutation cDNA	Protein	No. in the study/ total no. affected in the pedigree
1[a]	PGL001	c.112 C→T	R38X	1/7
2[b]	PGL003	c.305 A→T	H102L	4/11
3[b]	PGL005	c.242 C→T	P81L	1/15
4[b]	PGL007	c.242 C→T	P81L	5/9
5[b]	PGL008	c.106 C→T	Q36X	10/26
6[b]	PGL011	c.112 C→T	R38X	5/9
7	PGL020	c.147–148insA	H50fsX68	1/2
8	PGL021	c.242 C→T	P81L	1/7
9[b]	PGL024	c.242 C→T	P81L	6/7
10[b]	PGL026	c.242 C→T	P81L	5/7
11[a]	PGL027	c.381–383delG	L128fsX134	1/3
12	PGL030	c.242 C→T	P81L	2/2
13	PGL031	c.242 C→T	P81L	1/2
14[a]	PGL033	c.242 C→T	P81L	1/4
15	PGL034	c.242 C→T	P81L	2/5
16	PGL035	c.129 G→A	W43X	5/13
17	PGL041	c.242 C→T	P81L	1/2
18	PGL044	c.242 C→T	P81L	1/2
19	PGL045	IVS 2–1G→T	Aberrant splicing	1/3
20[a]	PGLSp52	c.242 C→T	P81L	1/6
21[a]	PGLSp59	c.242 C→T	P81L	1/1
22[a]	PGLSp64	c.325 C→T	Q109X	1/2
23[a]	PGLSp77	c.242 C→T	P81L	1/2

[a]Mutations described in Baysal et al. (2002)
[b]Mutations described in Baysal et al. (2000)

Results

PGL1 subjects and the mutations

The 58 individuals analyzed come from 23 families with *SDHD* mutations. The mutational status of 14 families has previously been described, whereas nine of them are first described here (Table 1). The families had a total of nine distinct mutations: two missense, six nonsense, and one splice site mutation. All mutations co-segregated with the disease phenotype and were not observed in at least 200 control chromosomes. The maternal imprinting rule was not violated in any of the new families: all observed disease transmissions occurred in father-child pairs. Thirty-three subjects had P81L and H102L missense mutations. The remaining 25 subjects had diverse mutations including IVS2–1G→T, a splice site mutation that causes skipping of exon 3 (Renard et al. 2003), and nonsense mutations Q36X, R38X, W43X, H50fsX68, Q109X, and L128fsX134. The two missense mutations, P81L and H102L, are predicted to yield a complete cybS protein of 159 amino acid with only a single amino acid residue change. Enzymatic studies in *Caenorhabditis elegans* (Ishii et al. 1998) and humans (Gimenez-Roqueplo et al. 2001) suggest that, whereas missense variants of regulatory subunits of MTCII retain the ability to be incorporated into the heterotetrameric complex, nonsense/splicing variants lead to the dissolution of the whole MTCII. The nonsense/splicing mutations are predicted to produce severely truncated/deleted and functionally null proteins. Furthermore, Q36X, R38X, W43X, and H50fsX68 mutations affect the mitochondrial signal peptide and are predicted to code for the absence of the mature protein.

The most common mutation, P81L, was present in 14 of the 23 (~61%) families. P81L has been implicated both as a founder and a recurrent mutation among the US families (Baysal et al. 2002). Joint haplotype analyses of P81L-carrying families indicated that mutations in families PGL005, PGL33, PGLSp77, PGL44, and PGLSp59 occurred on haplotypes distinct from the founder haplotype (data not shown), thus suggesting independent origins in five of 14 families (~36%). The remaining nine P81L families carried the founder mutation (~64%).

Phenotype at first clinical diagnosis

Tumor multiplicity

The phenotype at the first clinical diagnosis was assessed by analysis of tumor multiplicity at ACD and by ASO. The variables tested for their effects on tumor multiplicity

Table 2 Tumor multiplicity versus altitude at the first diagnostic evaluation (mean ± SE)

	0–1 Tumor (n=26)	Multiple tumors (n=32)	All (n=58)	NP P-value[a]
Age at symptom onset [ASO] (years)	32.6±2.9[b]	29.0±2.1	30.6±1.7[b]	0.25
Age at clinical diagnosis [ACD] (years)	33.8±2.8	34.5±2.2	34.2±1.7	0.30
Altitude [h(ACD)] (m)	200.9±22.0	452.8±80.0	340.0±47.8	0.0487
Altitude-year [h(ACD)×ACD] (m × year)	6,564±922	15,625±2,774	11,585±1,682	0.0117
Missense/nonsense-splicing	16/10	17/15	33/25	0.35

[a]Non-parametric (NP) tests, one-sided, comparing "0–1 Tumor" with "Multiple tumors"
[b]Excluding one subject without tumor

and ASO were mutation class (nonsense/splicing and missense), average altitude, and total altitude-years that the subjects were exposed to until ACD or ASO. The clinical diagnoses of all subjects were made at an average age of 34.2 years (range: 8–60 years; Table 2). Thirty-two subjects had multiple tumors (range: 2–5, median=2, mean=2.5), 25 had single tumors, and one at-risk carrier subject did not have any tumors at their first clinical evaluation. Of all detected paraganglioma tumors, CB tumors were the most common tumor type (~66%), followed by jugular (~7.5%), vagal paragangliomas (~5.6%), PHEOs (~5.6%), tympanic (3.7%), and other head and neck paragangliomas (~11%). Unilateral or bilateral carotid body tumors were present in 84.5% of the subjects.

Because PGL1 shows an age-dependent penetrance (Heutink et al. 1992), we asked whether differences in ACD explained why some subjects had single tumors, whereas others had multiple tumors. A comparison of ACD showed no significant difference between the groups with single or multiple tumors (Table 2). Thus, differences in ACD did not explain why multiple tumors were detected in certain subjects. An analysis of mutation type revealed that the number of missense and nonsense/splicing mutations were comparable between the groups with single tumor and multiple tumors, suggesting that mutation type did not affect tumor multiplicity either (Table 2). Because high altitude was implicated in the pathogenesis of sporadic tumors, we compared the average altitudes, h(ACD) with a range of 23 m to 1,382 m, between the groups with single and multiple tumors. We found that each subject lived at, on average, 2.6 (median=2, range: 1–8) different geographical locations for an average duration of 13 years (range: 0.5–60) per location by the ACD. Analyses of h(ACD)s revealed that subjects who had multiple tumors lived at significantly higher altitudes prior to their first diagnosis than the group with single tumors (Table 2). The difference was more significant when the comparison was made by using total altitude-years, h(ACD)×ACD, until first clinical diagnoses, suggesting that total altitude-years was a better predictor of developing multiple tumors.

Age at symptom onset

ASO did not differ significantly between the single-tumor- and multiple-tumor-harboring subjects at first clinical diagnosis (Table 2), suggesting that subjects with multiple tumors were not more likely to have an earlier symptom onset. We also tested whether altitude could affect the ASO and found only a weak negative correlation between the average altitude lived until the ASO, h(ASO), and the ASO (estimate for the Pearson coefficient of correlation, r=–0.124, P=0.178, one-sided). These results suggested that altitude, which influences tumor multiplicity significantly, did not have a statistically significant effect on ASO.

To test the effect of mutation class on ASO, we compared the missense- and nonsense/splicing-mutation-harboring groups. This analysis revealed that subjects with nonsense/splicing mutations developed symptoms 8.5 years earlier than did those with missense mutations (Table 3), revealing a significant difference. The earlier ASO in the nonsense/splicing mutation carriers also led to their clinical diagnosis at significantly earlier ages. The average altitude that the subjects lived at until ASO, h(ASO), was not different between the missense and nonsense/splicing mutation carriers, suggesting that altitude was not a significantly confounding factor in the observed difference in ASO between the nonsense/splicing and missense mutation carriers.

Lifetime phenotype

Tumor multiplicity

Analyses of the lifetime phenotypic data available for 57 subjects revealed that 14 subjects who had single tumors at their first clinical diagnoses went on to develop additional tumors later in life. The additional tumors were clinically detected on average 10.1 years (range: 2–21 years) after discovery of the first tumor. However, 12 other subjects, including the one asymptomatic subject, did not de-

Table 3 Mutation type and age at symptom onset (mean ± SE)

	Missense (n=33)	Nonsense/ splicing (n=25)	All (n=58)	NP P-value[a]
Age at symptom onset (years)	34.3±2.2[b]	25.8±2.6	30.6±1.7[b]	0.0118
Age at diagnosis (years)	37.8±2.1	29.5±2.7	34.2±1.7	0.017
Average altitude [h(ASO)] (m)	272.4±42.0[b]	421.9±97.4	338.0±49.3[b]	0.71

[a]Non-parametric Mann-Whitney U test, two-sided, compares the "Missense" and "Nonsense" groups
[b]Excluding one subject without tumor

Table 4 Altitude and tumor multiplicity in lifetime (mean ± SE)

	0–1 Tumor (*n*=12)	Multiple tumors (*n*=45)	All (*n*=57)	NP *P*-value[a]
Current age (CA)	54.7±6.2	50.8±2.0	51.6±2.0	0.39
Altitude [h(CA)] (m)	195.2±31.6	374.1±58.9	336.5±478	0.128
Altitude × year [h(CA)×CA] (m×year)	9,109±1,280	18,139±2,799	16,237±2,273	0.105
Missense/nonsense-splicing	7/5	25/20	32/25	0.56

[a]Non-parametric (NP) *P*-value, one-sided, comparing the "0–1 tumor" and "multiple tumor" groups

Table 5 Altitude and pheochromocytoma and malignancy development in lifetime (mean ± SE)

	Pheochromocytoma (*n*=6)	No pheochromocytoma (*n*=51)	NP *P*-value[a]	Malignancy (*n*=4)	No malignancy (*n*=53)	NP *P*-value[a]
Current age (years)	47.8±3.5	52.0±2.2	0.27	44.9±1.83	52.1±2.1	0.17
Altitude [h(CA)] (m)	863.2±244.6	274.5±37.8	0.013	282.4±107.13	340.6±50.9	0.49
Altitude × year [h(CA)×CA]	42,472±12,220	13,151±1,704	0.026	12,554±4,765	16,515±2,422	0.46
Missense/nonsense-splicing	1/5[b]	31/20	0.052	3/1	29/24	0.40

[a]Non-parametric tests (NP), one-sided, comparing "Pheochromocytoma" and "No pheochromocytoma" groups, and "Malignancy" and "No malignancy" groups, respectively

[b]Mutations associated with pheochromocytomas include W43X (n=4), L128fsX134 and P81L

velop further tumors. The 12 subjects who remained with zero tumor and one tumor in their lifetime continued to reside at lower altitudes and were exposed to lower altitude-years compared with the other 45 subjects who developed multiple tumors (range: 2–7, mean=3.2, median=3). However, the differences were not statistically significant (Table 4). The mutation type, again, did not have an effect on developing multiple tumors in lifetime.

PHEO, malignancy, and nonparaganglionic tumors

PHEOs are variable components of the PGL1 phenotype, and the factors that predispose to their development in PGL1 are unknown. The lifetime phenotypic analyses revealed development of PHEOs in six subjects (~10.5%). None of the subjects had PHEOs without the development of HNPs. Five subjects with PHEOs also had multiple HNPs, and one had a single HNP. The subjects with PHEOs were found to live at significantly higher average altitudes and were exposed to significantly higher altitude-years in their lifetime than those who did not develop these tumors. There was also a marginally significant association between nonsense mutations and PHEO development in PGL1 (Table 5). Four subjects (~6.9%) developed malignant tumors as evidenced by distant metastasis or local invasion. However, malignancy was not associated with either altitude or mutation type. Several subjects also reported as developing nonparaganglionic tumors, including ependymoma, melanoma, osteoid, and soft tissue tumors, and uterine fibroids, each of which developed in different subjects.

Comparison of population-weighted altitudes of the US and The Netherlands

We asked whether altitude could also account for some of the distinct population genetic characteristics of PGL1 in the central western Netherlands and compared the population-weighted altitudes of The Netherlands and the USA. For the USA, we used two approximations, one (called USA25) in which the lower boundary of the rectangle lay at 25° north and another (called USA30) in which the lower boundary of the rectangle lay at 30° north. The Florida Peninsula and much of northern Mexico were included in approximation USA25. Much of northern Mexico, the Florida Peninsula, and the Mississippi Delta were excluded in approximation USA30. Both approximations had an upper boundary at 49° north, an eastern boundary at 67° west, and a western boundary at 125° west. Box USA25 contained 66,204 data units with a total population of 276,816,522. Box USA30 contained 61,411 data units with a total population of 247,495,950. For comparison, the United Nations Population Division estimated, in 1997, that the total population of the USA in 1990 was 254,106,000.

For the central western portion of The Netherlands, we used a box (called NLD) with a lower boundary at 51.5° north, an upper boundary at 53.5° north, a western boundary at 3° east, and an eastern boundary at 6° east. Box NLD contained 425 data units, including Leiden and Rotterdam where *SDHD* founder effects were detected, with a total population of 10,138,215. For comparison, the United Nations Population Division estimated, in 1997, that the total population of The Netherlands in 1990 was 14,952,000.

These calculations revealed that the population-weighted mean elevation of US in 1990 was approximately 260 m and that of NLD was approximately 2 m (Table 6). For comparison, in 1994, the median person in the world lived

Table 6 Population-weighted mean elevations of the US and central-western Netherlands in 1990

Box	Mean elevation (m)	Standard deviation of elevation (m)
USA25	262.3	372.8
USA30	258.8	347.1
NLD	2.3	9.4

at an elevation of 194 m above sea level (Cohen and Small 1998). Comparison of the population-weighted mean elevations of the USA and NLD by using the *t*-test (with unequal variances) revealed a *P*-value that was essentially 0 (vanishingly small).

Discussion

We have shown that PGL1 subjects with nonsense/splicing *SDHD* mutations develop symptoms at earlier ages than those with missense mutations and that those with multiple tumors at the time of first diagnosis live at higher altitudes and are exposed to higher altitude-years. We have also shown that PHEOs, a variable component of the PGL1 phenotype, preferentially develop in subjects who live at higher altitudes and who have nonsense/splicing germ-line mutations. Previously, most individuals and families with PGL1 and PHEOs have also been found to carry germ-line nonsense/splicing mutations in *SDHD*. These mutations include IVS1+2T→G, A13 fs, W5X, C11X, R22X, S32 fs, R38X, and Q121X (Gimm et al. 2000; Astuti et al. 2001a; Neumann et al. 2002). However, these hormone-secreting tumors have been reported in only three of 120 Dutch paraganglioma patients (Dannenberg et al. 2002; van Schothorst et al. 1998), who overwhelmingly carry missense founder mutations. Our analysis of population-weighted altitudes has revealed unusually low elevations in the central western Netherlands, where three PGL1 founder mutations are observed in increased frequency among nonfamilial cases. These findings suggest that higher altitudes facilitate the development of PGL1 tumors, including PHEOs, and provide a rare example of an environmental factor that is significantly associated with the penetrance, the expressivity, and the population genetics of an inherited tumor syndrome. Notably, the *VHL* gene mutated in Von-Hippel Lindau syndrome, another cause of heritable PHEOs, is also proposed to function in oxygen homeostasis in the normoxic degradation of hypoxia-inducible factor 1 alpha (Maxwell et al. 1999). However, to our knowledge, no gene-environment studies for VHL syndrome have ever been conducted.

To explain the association of tumor multiplicity with altitude and of the mutation type with age at onset, we suggest that the cellular proliferation in PGL1 is initially induced by a defect in oxygen sensing. This defect is exacerbated at moderately higher altitudes, which leads to an increased number of actively dividing cells and to an increased likelihood of a second-hit somatic mutation. Therefore, living at higher altitudes is expected to facilitate the development of independent tumor foci that develop clonally following the second-hit mutation. Whereas the initial pre-second-hit growth of paraganglionic cells may be dependent more on the environment (hypoxia), once the second-hit removes the normal nonmutant allele, the post-second-hit growth rate should be determined primarily by the residual function of the remaining (mutant) allele. Subsequently, nonsense/splicing mutations that disrupt structural integrity and are predicted to lead to complete dissolution of MTCII may be associated with a faster post-second-hit growth rate and with an earlier symptom onset. Thus, two events determine ASO: the timing of the initial second-hit event, which is influenced by altitude, and the residual function of the mutant allele following the second-hit, which is influenced by the inherited mutation type. Because nonsense/splicing mutation, but not altitude, is significantly associated with earlier symptom onset in our subjects, we suggest that the rate of tumor growth among our subjects is primarily dictated by the residual function of the mutant allele once the second-hit somatic mutation occurs.

Our results suggest that higher atmospheric oxygen pressures and the missense mutations jointly contribute to the frequent detection of founder mutations in nonfamilial cases and possibly to the high incidence of PGL1 in The Netherlands. Genomic imprinting at *PGL1* may also confound the observation of many nonfamilial cases carrying germ-line founder mutations. The mutations may pass through maternal lineages over many generations before the mutation is expressed after a paternal transmission. However, there is no evidence or rationale to postulate that such peculiar transmissions are more prevalent in The Netherlands than in the USA. A more likely explanation for the observation of significantly higher number of nonfamilial cases carrying founder mutations is that they have at-risk relatives who remain clinically unaffected. Our study suggests that the very low altitudes in The Netherlands may significantly delay or prevent the occurrence of second-hit mutations and that the post-second-hit growth rate would be slow because of the missense nature of the founder mutations.

Because of the mortality and morbidity associated with PGL1 in the reproductive period, as partly evidenced by the paucity of founder mutations elsewhere in the world, it is plausible that *SDHD* mutations are under selective pressure. Thus, negative selection that acts through the disease phenotype may be relaxed in The Netherlands because of reduced penetrance caused by the unusually low altitudes, leading the Dutch missense founder mutations to behave like neutral mutations without significant selective constraints. Although many people around the world also live at low elevations, altitude variations within countries are likely to play a role in determining cumulative hypoxic exposure. For example, when subjects are mobilized, e.g., for professional or touristic reasons, within a country that shows great variations in altitude, such as the USA, they will be exposed to the hypoxia of higher altitudes, in proportion to the degree of elevation and the du-

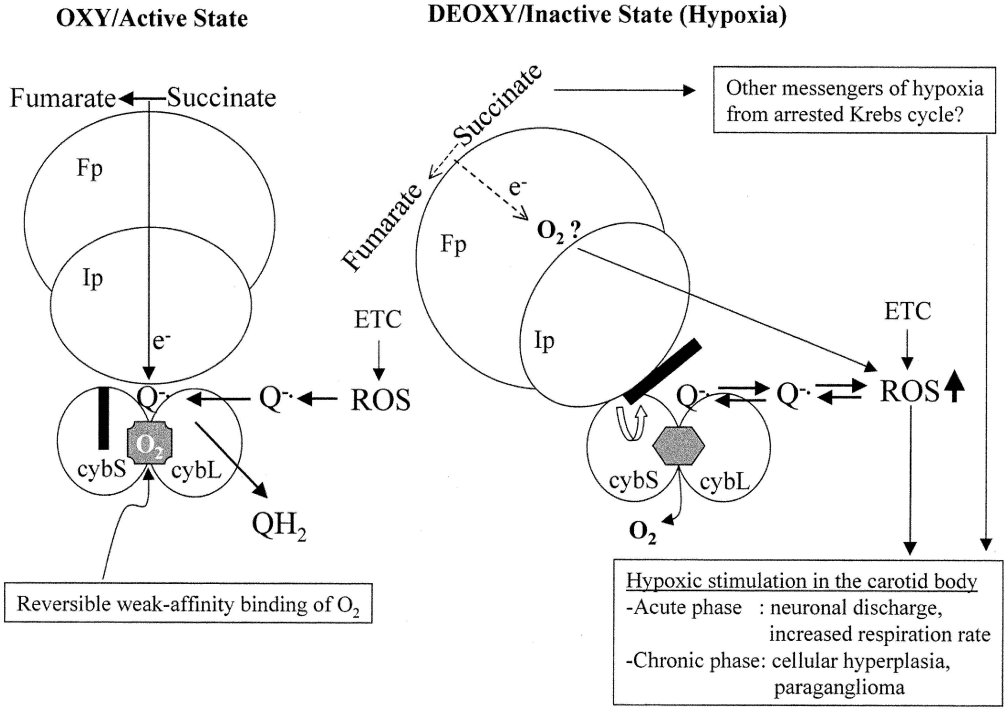

Fig. 1 A highly schematic, hypothetical model for oxygen-sensing by the mitochondrial complex II (MTCII) and its disturbance in PGL. MTCII is composed of four subunits: Fp flavoprotein catalytic subunit encoded by *SDHA*, Ip iron-sulfur catalytic subunit encoded by *SDHB*, cybS the small subunit in cytochrome b encoded by *SDHD*, cybL the large subunit in cytochrome b encoded by *SDHC*. MTCII may have two configurations regulated by weak affinity binding of molecular oxygen by the heme in the cytochrome b. *Left* The OXY configuration is associated with reduced reactive oxygen species (ROS) levels, which might be achieved through two pathways: the ROS produced by the electron transport chain (*ETC*) is effectively removed via reduction of ubisemiquinone ($Q^{-\cdot}$) to ubiquinol (QH_2) by means of the electrons (e^-) derived from the oxidation of succinate to fumarate in the Krebs cycle, and/or the complete transfer of the electrons through MTCII prevents their accumulation in the flavoprotein and iron-sulfur subunits, which might lead to ROS production through chemical interaction with molecular oxygen (Messner and Imlay 2002). *Right* In the DEOXY state (hypoxia), the electron transfer along the axis of MTCII is disrupted (*dashed lines*) because of a conformational change depicted symbolically by the movement of a solid black bar (curved arrow) caused by the dissociation of oxygen, which in turn leads to an accumulation of ROS through the operation of one or both mechanisms mentioned above. The increased ROS subsequently activates hypoxia-responsive pathways. The *SDHD*, *SDHC*, and *SDHB* inactivating mutations in PGL are predicted to cause constitutive activation of the ROS-producing pathways and of the hypoxic pathways, eventually leading to carotid body (CB) hyperplasia and the development of a tumor. This simple model is compatible with the existing data from the genetics of PGL, the *C.elegans mev-1* model, and our current study and also allows for the possibility of other secondary messengers of hypoxia that may be derived from blockage of the Krebs cycle at the succinate dehydrogenase step (*plaque, hexagon* different conformations of the putative oxygen-binding heme-domain)

ration of stay, which may trigger neoplastic cell division in PGL1. In contrast, low altitudes combined with the small altitude variations in The Netherlands may not generate enough cumulative hypoxic exposure to initiate tumor development.

In addition to altitude, short-term weather fluctuations, seasonal climatic fluctuations, and geographic fluctuations (differences between latitudes, differences between continents and oceans) may influence atmospheric pressure. As our data pertain to years of exposure, the subjects' experiences are averaged over short-term weather fluctuations and seasonal fluctuations at a given place. All subjects live on land, and so differences between oceans and continents can also be ignored. However, differences between latitudes require attention. Our data are drawn from latitudes ranging from 25° or 30° north to 49° north for the USA and between 51.5° and 53.5° north for The Netherlands. According to West (1996), the impact of latitude at sea level is very small. In July, between 30° north and 60° north, model atmospheric pressures range over 2 Torr (from 758 Torr at 60° north to 760 Torr at 30° north). In January, the range is 6 Torr (from 760 Torr at 60° north to 766 Torr at 30° north). Thus, the annual average varies by no more than 6 Torr over the range from 30° north to 60° north, all at zero altitude. Similarly, at 1 km altitude, for the range from 30° north to 45° north, the range of atmospheric pressures in January is no more than 7 Torr (from 673 Torr at 45° north to 680 Torr at 30° north). Thus, in the latitudes relevant to our study and over the range from sea level to 1 km altitude, the annual average atmospheric pressure does not vary by more than 6 or 7 Torr. In Table 2, the altitude for 0–1 tumors, namely 200.9 m, corresponds to 742.7 Torr, and the altitude for multiple tumors, namely 452.8 m, corresponds to 722.0 Torr according to the Model Atmosphere equation. The difference of more than 20 Torr greatly exceeds the variation in annual averages attributable to latitude in the range relevant to our study. The same conclusion can be made for the significantly different altitudes in Table 5. In summary, we conclude that al-

titude is the principal factor determining atmospheric pressures exposed to the PGL1 subjects in our study and that the confounding effects of weather, climate, or latitude are probably negligible.

If the association of higher atmospheric pressures with the delayed appearance of PGL1 tumors is confirmed, it has important implications for the clinical management of at-risk carrier individuals and for the genetic counseling of families. Our results suggest that living at low altitudes, close to sea level, may be associated with a milder phenotype. However, it should be emphasized that this association is not absolute and that there are PGL1 subjects with multiple tumors who live at very low altitudes. Thus, additional genetic modifiers are likely to play roles in affecting tumor multiplicity. Whether therapeutic exposure to hyperbaric oxygen could further improve the PGL1 phenotype is unknown and should be first explored in an animal model.

Because the altitudes associated with multiple tumor development in PGL1 are not as high as those that cause sporadic paragangliomas, such as those observed in the Andean mountains, the paraganglia in subjects with *SDHD* mutations are likely to become more susceptible to hypoxia-induced tumorigenesis because of the reduced number of wild-type complex IIs. The lack of disease phenotype after maternal transmission (genomic imprinting) and the observation of LOH targeting the maternal allele in PGL1 tumors can also be explained if most, but not all, of cybS is contributed by the paternal allele in human paraganglia. However, the exact molecular mechanisms of the way in which cybS could be involved in responding to altered atmospheric oxygen levels are unknown. A homozygous loss-of-function mutation in the ortholog of *SDHC*, which encodes cybL– (the large subunit of cytochrome b in MTCII) in the *C. elegans mev-1* mutant, causes oxygen hypersensitivity, oxidative stress, and premature aging because of an abundance of reactive oxygen species (ROS) and leads to lactic acidosis, a finding suggestive of Krebs cycle impairment (Ishii et al. 1998; Senoo-Matsuda et al. 2001). These findings suggest that MTCII has a critical role in the regulation of ROS levels, and that this regulation is mechanistically linked to the transfer of electrons derived from the oxidation of succinate to fumarate in the Krebs cycle to ubiquinone. How can these observations in *C. elegans* be reconciled with our findings that higher altitudes facilitate tumorigenesis in PGL1?

According to a mitochondrial model of cellular oxygen sensing, hypoxia increases mitochondrial ROS levels, which subsequently activate downstream targets to adapt to hypoxia (Chandel and Schumacker 2000). It is plausible that MTCII is critically involved in the regulation of hypoxia-induced ROS levels, and that this regulation is primarily controlled by weakly bound molecular oxygen. A weak and reversible binding of molecular oxygen may be the rate-limiting step for the transition of MTCII to the active enzymatic conformation that is associated with reduced ROS levels (Fig. 1). At moderately high altitudes, molecular oxygen may normally dissociate from a small fraction of wild-type MTCIIs. However, because the number of wild-type MTCIIs is reduced in PGL1 because of heterozygous mutations, dissociation of molecular oxygen from a small fraction of wild-type MTCIIs may increase ROS to a level comparable with that observed at high altitudes in normal individuals. Under both conditions, the number of oxygen-binding wild-type MTCIIs that are associated with low ROS levels would fall below a critical threshold, and the subsequent increase in ROS would help trigger a hypoxic response. The primary candidate molecular domain for the weak-affinity binding of molecular oxygen is the heme in cytochrome b. The recent discovery of the structure of bacterial SDH indicates that this heme does not participate in the electron transfer pathway and it has been suggested as an electron sink that prevents the inadvertent leakage of electrons. However, its precise function in MTCII remains unclear (Hederstedt 2003; Yankovskaya et al. 2003). Consistent with our proposal that this heme may be an oxygen-sensor, an evolutionarily conserved histidine in cybS, which is thought to provide an axial ligand for the heme, is altered by the H102L mutation in an extended PGL1 family (Table 1). These hypotheses await further testing by genetic, biochemical, and structural studies of MTCII.

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References

Arias-Stella J, Valcarcel J (1976) Chief cell hyperplasia in the human carotid body at high altitudes; physiologic and pathologic significance. Hum Pathol 7:361–373

Astuti D, Douglas F, Lennard TW, Aligianis IA, Woodward ER, Evans DG, Eng C, Latif F, Maher ER (2001a) Germline SDHD mutation in familial phaeochromocytoma. Lancet 357:1181–1182

Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, Skoldberg F, Husebye ES, Eng C, Maher ER (2001b) Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet 69:49–54

Badenhop RF, Cherian S, Lord RS, Baysal BE, Taschner PE, Schofield PR (2001) Novel mutations in the SDHD gene in pedigrees with familial carotid body paraganglioma and sensorineural hearing loss. Genes Chromosome Cancer 31:255–263

Baysal BE (2001) Genetics of familial paragangliomas: past, present, and future. Otolaryngol Clin North Am 34:863–79

Baysal BE (2002) Hereditary paraganglioma targets diverse paraganglia. J Med Genet 39:617–622

Baysal BE, Farr JE, Rubinstein WS, Galus RA, Johnson KA, Aston CE, Myers EN, Johnson JT, Carrau R, Kirkpatrick SJ, Myssiorek D, Singh D, Saha S, Gollin SM, Evans GA, James MR, Richard CW III (1997) Fine mapping of an imprinted gene for familial nonchromaffin paragangliomas, on chromosome 11q23. Am J Hum Genet 60:121–132

Baysal BE, Schothorst EM van, Farr JE, Grashof P, Myssiorek D, Rubinstein WS, Taschner P, Cornelisse CJ, Devlin B, Devilee P, Richard CW (1999) Repositioning the hereditary paraganglioma critical region on chromosome band 11q23. Hum Genet 104:219–225

Baysal BE, Ferrell RE, Wilett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, Mey A van der, Taschner PEM, Rubinstein WS, Myers EN, Richard CW III, Cornelisse CJ, Devilee P, Devlin B (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287:848–851

Baysal BE, Willett-Brozick JE, Lawrence EC, Drovdlic CM, Savul SA, McLeod DR, Yee HA, Brackmann DE, Slattery WH III, Myers EN, Ferrell RE, Rubinstein WS (2002) Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas. J Med Genet 39: 178–183

Cascon A, Ruiz-Llorente S, Cebrian A, Telleria D, Rivero JC, Diez JJ, Lopez-Ibarra PJ, Jaunsolo MA, Benitez J, Robledo M (2002) Identification of novel SDHD mutations in patients with phaeochromocytoma and/or paraganglioma. Eur J Hum Genet 10:457–461

Chandel NS, Schumacker PT (2000) Cellular oxygen sensing by mitochondria: old questions, new insight. J Appl Physiol 88: 1880–1889

Cohen JE, Small C (1998) Hypsographic demography: the distribution of human population by altitude. Proc Natl Acad Sci USA 95:14009–14014

Cremers CW, De Monnink JP, Arts N, Joosten FB, Kremer H, Hoefsloot L (2002) Clinical report on the L95P mutation in a Dutch family with paraganglioma. Otol Neurotol 23:755–759

Dannenberg H, Dinjens WN, Abbou M, Van Urk H, Pauw BK, Mouwen D, Mooi WJ, Krijger RR de (2002) Frequent germline succinate dehydrogenase subunit D gene mutations in patients with apparently sporadic parasympathetic paraganglioma. Clin Cancer Res 8:2061–2066

Gimenez-Roqueplo AP, Favier J, Rustin P, Mourad JJ, Plouin PF, Corvol P, Rotig A, Jeunemaitre X (2001) The R22X mutation of the SDHD gene in hereditary paraganglioma abolishes the enzymatic activity of complex II in the mitochondrial respiratory chain and activates the hypoxia pathway. Am J Hum Genet 69:1186–1197

Gimm O, Armanios M, Dziema H, Neumann HP, Eng C (2000) Somatic and occult germ-line mutations in SDHD, a mitochondrial complex II gene, in nonfamilial pheochromocytoma. Cancer Res 60:6822–6825

Gonzalez C, Almaraz L, Obeso A, Rigual R (1994) Carotid body chemoreceptors: from natural stimuli to sensory discharges. Physiol Rev 74:829–898

Hederstedt L (2003) Structural biology. Complex II is complex too. Science 299:671–672

Heutink P, Mey AG van der, Sandkuijl LA, Gils AP van, Bardoel A, Breedveld GJ, Vliet M van, Ommen GJ van, Cornelisse CJ, Oostra BA, Weber JL, Devilee P (1992) A gene subject to genomic imprinting and responsible for hereditary paragangliomas maps to chromosome 11q23-qter. Hum Mol Genet 1: 7–10

Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K (1998) A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 394:694–697

Lancaster CRD (2002) Special issue on Bioenergetics. Biochim Biophys Acta 1320:1–176

Mariman ECM, Beersum SEC van, Cremers CWRJ, Baars FM van, Ropers HH (1993) Analysis of a second family with hereditary non-chromaffin paragangliomas locates the underlying gene at the proximal region of chromosome 11q. Hum Genet 91:357–361

Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399: 271–275

Messner KR, Imlay JA (2002) Mechanism of superoxide and hydrogen peroxide formation by fumarate reductase, succinate dehydrogenase, and aspartate oxidase. J Biol Chem 277:42563–42571

Mey AG van der, Maaswinkel-Mooy PD, Cornelisse CJ, Schmidt PH, Kamp JJ van de (1989) Genomic imprinting in hereditary glomus tumours: evidence for new genetic theory. Lancet II:1291–1294

Milunsky JM, Maher TA, Michels VV, Milunsky A (2001) Novel mutations and the emergence of a common mutation in the SDHD gene causing familial paraganglioma. Am J Med Genet 100:311–314

Neumann HP, Bausch B, McWhinney SR, Bender BU, Gimm O, Franke G, Schipper J, Klisch J, Altehoefer C, Zerres K, Januszewicz A, Smith WM, Munk R, Manz T, Glaesker S, Apel TW, Treier M, Reineke M, Walz MK, Hoang-Vu C, Brauckhoff M, Klein-Franke A, Klose P, Schmidt H, Maier-Woelfle M, Peczkowska M, Szmigielski C, Eng C (2002) Germline mutations in nonsyndromic pheochromocytoma. N Engl J Med 346:1459–1466

Niemann S, Muller U (2000) Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nat Genet 26:268–270

Renard L, Godfraind C, Boon LM, Vikkula M (2003) A novel mutation in the SDHD gene in a family with inherited paragangliomas-implications of genetic diagnosis for follow up and treatment. Head Neck 25:146–151

Schothorst EM van, Jansen JC, Grooters E, Prins DE, Wiersinga JJ, Mey AG van der, Ommen GJ van, Devilee P, Cornelisse CJ (1998) Founder effect at PGL1 in hereditary head and neck paraganglioma families from The Netherlands. Am J Hum Genet 63:468–473

Senoo-Matsuda N, Yasuda K, Tsuda M, Ohkubo T, Yoshimura S, Nakazawa H, Hartman PS, Ishii N (2001) A defect in the cytochrome b large subunit in complex II causes both superoxide anion overproduction and abnormal energy metabolism in *Caenorhabditis elegans*. J Biol Chem 276:41553–41558

Taschner PE, Jansen JC, Baysal BE, Bosch A, Rosenberg EH, Brocker-Vriends AH, Mey AG van der, Ommen GJ van, Cornelisse CJ, Devilee P (2001) Nearly all hereditary paragangliomas in The Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosome Cancer 31:274–281

West JB (1996) Prediction of barometric pressures at high altitude with the use of model atmospheres. J Appl Physiol 81:1850–1854

Yankovskaya V, Horsefield R, Tornroth S, Luna-Chavez C, Miyoshi H, Leger C, Byrne B, Cecchini G, Iwata S (2003) Architecture of succinate dehydrogenase and reactive oxygen species generation. Science 299:700–704