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Variations within oxygen-regulated gene expression in humans

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Brooks JT, Elvidge GP, Glenny L, Gleadle JM, Liu C, Ragoussis J, Smith TG, Talbot NP, Winchester L, Maxwell PH, Robbins PA. Variations within oxygen-regulated gene expression in humans. *J Appl Physiol* 106: 212–220, 2009. First published November 13, 2008; doi:10.1152/japplphysiol.90578.2008.—The effects of hypoxia on gene transcription are mainly mediated by a transcription factor complex termed hypoxia-inducible factor (HIF). Genetic manipulation of animals and studies of humans with rare hereditary disease have shown that modifying the HIF pathway affects systems-level physiological responses to hypoxia. It is, however, an open question whether variations in systems-level responses to hypoxia between individuals could arise from variations within the HIF system. This study sought to determine whether variations in the responsiveness of the HIF system at the cellular level could be detected between normal individuals. Peripheral blood lymphocytes (PBL) were isolated on three separate occasions from each of 10 healthy volunteers. After exposure of PBL to eight different oxygen tensions ranging from 20% to 0.1%, the expression levels of four HIF-regulated transcripts involved in different biological pathways were measured. The profile of expression of all four transcripts in PBL was related to oxygen tension in a curvilinear manner. Double logarithmic transformation of these data resulted in a linear relationship that allowed the response to be parameterized through a gradient and intercept. Analysis of variance (ANOVA) on these parameters showed that the level of between-subject variation in the gradients of the responses that was common across all four HIF-regulated transcripts was significant ($P = 0.008$). We conclude that statistically significant variation within the cellular response to hypoxia can be detected between normal humans. The common nature of the variability across all four HIF-regulated genes suggests that the source of this variation resides within the HIF system itself.

hypoxia-inducible factor; reverse transcription quantitative polymerase chain reaction; oxygen tension; peripheral blood lymphocytes

MAINTENANCE OF OXYGEN HOMEOSTASIS is crucial for the survival of aerobic organisms. Hypoxia induces a range of responses at both molecular and systemic physiological levels in order to maintain a sufficient supply of oxygen to cells. In addition, the response to localized hypoxia within tissues is important to the outcome of various disease states. For example, the local hypoxia resulting from coronary artery disease causes a beneficial angiogenic response in which collateral vessels are formed in order to bypass the area of stenosis in the diseased artery (6, 43). In cancer, cellular hypoxia is important in promoting angiogenesis and metastasis (13, 45).

Among humans, there is a considerable degree of variability in physiological responses to hypoxia. Interindividual and population-specific variability has been reported in the ventilatory (7, 23, 27, 41, 44, 55), pulmonary-vascular (5, 21), and erythropoietin (Epo) (8, 14, 20, 40, 51, 52) responses to hypoxia. Similarly, variation has also been reported in the response to hypoxia in different disease states. For example, variations in angiogenic responses to hypoxia may alter adaptive changes in coronary artery disease (6, 43) and susceptibility to diabetic retinopathy (1).

While these variations in systems-level responses to hypoxia are well characterized in humans, little is known about the variations in the cellular and molecular mechanisms that give rise to these differences. The oxygen-regulated transcription of genes is largely governed by a transcription control complex termed hypoxia-inducible factor (HIF). HIF is part of a well-understood pathway linking changes in the concentration of molecular oxygen to alterations in transcription of a broad range of genes (42). Genetic manipulation of this system in mice and a rare genetic disease (Chuvash polycythemia) that results in partial HIF activation in humans have both shown that this system plays an important role in determining a number of physiological characteristics including the erythropoietic (2, 3, 12, 22, 39, 46, 54), ventilatory (28, 48), and cardiopulmonary (9, 10, 47, 48, 54) responses to hypoxia. Our hypothesis is that variations within the HIF transcription system underlie at least part of the variation in systems-level responses to hypoxia among normal human individuals. The aim of this study was to determine whether variations within the HIF transcription system can be detected between normal individuals. If such variations can be detected, then this would provide a platform for future studies in humans to explore links between functional variation within the HIF system and variation within integrated physiological responses to hypoxia.

The experimental approach adopted was to compare the between-subject variation in the response of the HIF system to changes in oxygen tension with the within-subject variation in these responses. One significant problem is that the α-subunit of HIF (HIF-α) is a highly labile protein present in low quantities within the cell. As a consequence, assay-to-assay variability in the quantification of HIF-α protein would almost certainly exceed any naturally occurring differences in expression between individuals. For this reason, rather than attempting to quantify directly the variation in the level of HIF-α protein with varying oxygen tension, we chose to quantify variations in the level of mRNA of selected HIF-regulated transcripts. Since these downstream products of HIF regulation are almost certainly affected by genetic variation between indi-

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Table 1. *Physical characteristics of participants*

Subject No.	Age, yr	Sex	Body Mass Index, kg/m^2	Ethnicity
1096	27	M	21.6	Caucasian
1229	24	M	19.4	Caucasian
1286	23	M	19.4	Caucasian
1490	21	M	23.1	Asian
1506	27	F	20.2	Caucasian
1508	38	F	20.9	Caucasian
1513	30	F	22.5	Caucasian
1522	26	M	26.5	Caucasian
1973	30	M	21	Caucasian
2000	41	M	22.9	Caucasian
Mean	29		21.8	
SD	7		2.1	

viduals besides that within the HIF system, we chose to examine four different mRNAs that encode proteins with functions in four different biological pathways. Variation in the amplitude of responses to hypoxia that is common across genes is highly likely to arise from variation in the HIF system.

Peripheral blood lymphocytes (PBL) represent a cell type relatively easily obtainable from human subjects for experimental study in which induction of HIF by hypoxia can be observed (32). In this study we measured the expression of four selected HIF-regulated mRNA transcripts in human PBL under oxygen tensions ranging from 20% to 0.1%. The results were analyzed by using analysis of variance (ANOVA) to identify the between-subject variation that was common across the four different transcripts and compare this with the level of within-subject variation.

MATERIALS AND METHODS

Overall experimental design. An initial study identified HIF-regulated genes that were expressed in PBL and showed a high level of induction by hypoxia. After this, blood was taken on three separate occasions from each of 10 subjects. PBL were isolated, aliquoted, and incubated under a range of oxygen tensions. The abundance of four different HIF-regulated transcripts, together with an internal control transcript, was quantified with reverse transcription quantitative PCR (RTqPCR). The results of transcript expression were parameterized and then subjected to ANOVA using SPSS (SPSS, Chicago, IL) to determine whether significant interindividual variation in transcript expression was present among the subjects that was common across the four genes.

Selection of HIF-regulated genes for assessment of HIF system variability. We previously showed (48) that the HIF-regulated gene transcripts aldolase C (*ALDC*) and vascular endothelial growth factor (*VEGF*) show robust hypoxic induction over a range of hypoxic stimuli in PBL. To identify additional HIF-regulated gene transcripts showing hypoxic induction in PBL, we performed three independent paired 20-h incubations at 20% and 1% oxygen tensions of six PBL samples from one subject as described previously (48). The six RNA samples from these experiments were analyzed for transcript expression with a Sentrix-6 BeadChip platform (Illumina). After analyses of

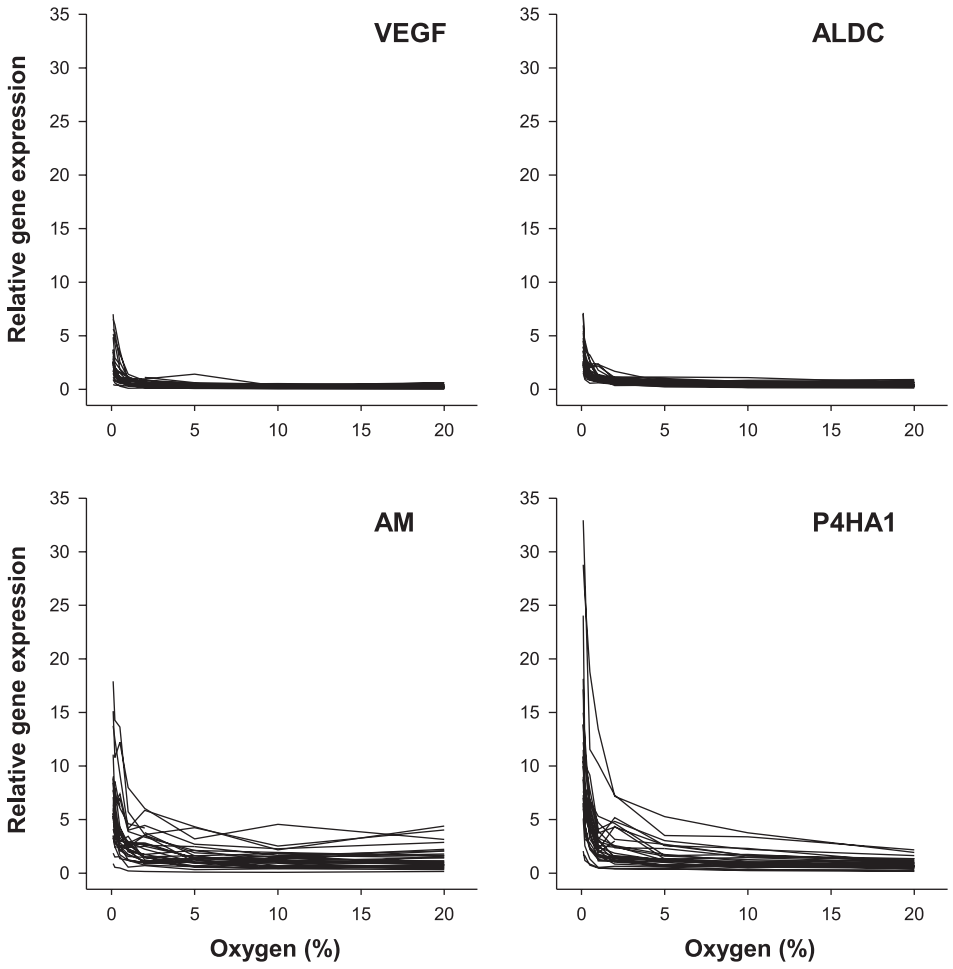

Fig. 1. Relative expression of vascular endothelial growth factor (*VEGF*), aldolase C (*ALDC*), adrenomedullin (*AM*), and prolyl-4-hydroxylase α1 (*P4HA1*) over a range of oxygen tensions in peripheral blood lymphocytes. Data are for 3 repeat experiments on each of 10 human subjects.

transcript expression, the gene-specific signal intensities in the euoxic samples were compared with those of the hypoxic samples. The average ratios of the hypoxia to euoxia signal intensities from the three repeat experiments together with all the results of the microarray analyses have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus database under accession number GSE7833. From the results of this microarray screening process we selected two further HIF-regulated gene transcripts, prolyl-4-hydroxylase α1 (*P4HA1*) (50) and adrenomedullin (*AM*) (17, 34), to be used in the assessment of variability of the HIF system among human subjects. *AM* and *P4HA1* were both among the 100 transcripts showing the highest levels of hypoxic induction out of 47,000 transcripts interrogated by the BeadChip arrays.

Subjects recruited for main study. Ten healthy human subjects were recruited for the study. Their physical characteristics are given in Table 1. Subjects gave informed consent before donating samples of venous blood. The study conformed to the principles of the Declaration of Helsinki and had approval from the Oxfordshire Clinical Research Ethics Committee. All subjects were healthy, although one female subject (*1508*) was noted to be mildly iron deficient with low serum iron and ferritin levels. This subject did not appear unusual with respect to any of the experimental results.

Determination of hypoxically regulated gene expression in PBL over a range of oxygen tensions. PBL from each subject were isolated, aliquoted, and incubated as previously described (48). In brief, 40 ml of venous blood was centrifuged over density gradient medium (Ficoll-Paque Plus, Amersham Biosciences, Chalfont, UK) to isolate the mononuclear cells. These were then incubated at 37°C for 30 min over a hydrophilic membrane to induce cellular adhesion by the monocytes, leaving a supernatant containing the PBL. The PBL were aliquoted into gas-permeable tissue culture dishes and incubated at eight different oxygen tensions (20%, 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1%) for 20 h. The incubations were achieved by suspending each tissue culture dish inside a large glass container that was flushed with the relevant gas mixture for 10 min before the incubation period. The composition of the gas before and after incubation was checked to ensure it had remained constant by using a paramagnetic oxygen analyzer that had been calibrated with a standard gas mixture. After completion of the incubations, total RNA was harvested from the PBL by a column-based extraction method (RNAqueous-4PCR, catalog no. 1914, Ambion). RTqPCR using TaqMan primers and probes was used to determine the expression of *ALDC*, *VEGF*, *AM*, and *P4HA1* together with β_2-microglobulin (*β2MG*), which had shown no induction in hypoxia in the preliminary experiments and was therefore used as an internal control, as described previously (48). A calibrator sample of complementary DNA (cDNA) was run with each set of qPCR reactions. In one subject (*1286*), the RNA samples of one repeat experiment at 5% O_2 and another repeat experiment at 1% O_2 gave uninterpretable results with RTqPCR and were thus excluded from further analyses. Relative HIF-regulated gene expression was quantified by a method described by Pfaffl (38).

Statistical analysis. Variations in gene expression with oxygen tension from all of the experiments were first examined on linear and double logarithmic plots. Because the responses appeared linear in the double logarithmic plots, the results for each experiment on each subject could be parameterized by linear regression on these values.

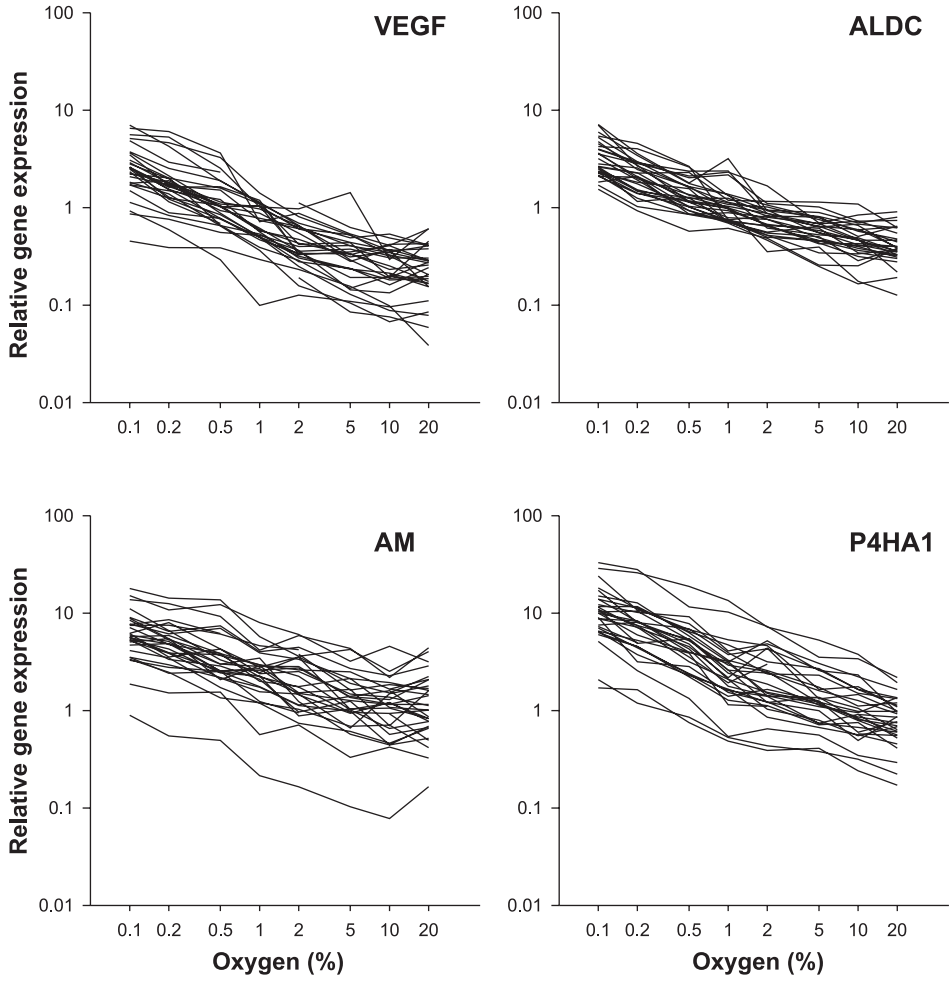

Fig. 2. Relative expression in peripheral blood lymphocytes of *VEGF*, *ALDC*, *AM*, and *P4HA1* over a range of oxygen tensions plotted on a double logarithmic scale. Data are as for Fig. 1.

This provided one value for the gradient of the response and another value for the intercept (expression at 10% oxygen). Next, in order to weight equally the results of the different genes studied within the statistical analysis, the slopes and intercepts for each gene were scaled so that each had a variance of unity. The main statistical analysis was undertaken with ANOVA. The principal factors used in the analysis were "subject" and "gene." In addition to the principal factors, the interactive factors of "subject" with "gene" (reflecting the variation between subjects that varies between different genes) and "subject" with "experimental repeat" (reflecting the variation between individual experiments) were also included in the ANOVA. For the purposes of generating the appropriate error terms, "gene" was considered as a fixed factor and "subject" and "experimental repeat" were considered as random factors. The significance of the "subject" factor represents the main outcome measure of this study, because it reflects the variation between subjects that is common across the four HIF-regulated genes under study.

RESULTS

To show the overall variation in transcript level with oxygen tension in human subjects, the data from all of the repeat experiments at each oxygen tension are plotted for each gene in Fig. 1 (where *β2MG* has been used as an internal control). All four genes show a similar response profile to oxygen; there is a relatively small increase in gene expression from 20% to 2% oxygen and a very large increase from 2% to 0.1% oxygen, with no indication of a plateau in the response being reached. In contrast, the threshold cycle values from the RTqPCR for the internal control gene, *β2MG*, showed no relationship with hypoxia. It is also clear from Fig. 1 that there is inherently more variation in the gene expression measurements at lower oxygen tensions. When the same values are plotted on a double logarithmic scale as shown in Fig. 2, the relationship between transcript expression and oxygen tension appears to be linear, with a similar degree of variation in gene expression measurements occurring at each oxygen tension. These appearances are further confirmed in Fig. 3, where the mean values for gene expression at each value for oxygen tension are plotted in a double logarithmic format together with the intersubject standard deviations. The linear nature of the response when plotted in double logarithmic format is apparent from the simple linear regressions shown for each of the genes.

The observations above indicate that it is possible to parameterize the responses of individual experiments by simple linear regression on the logarithmic values for gene expression and oxygen tension. Examples of this are shown in Fig. 4 for two subjects; *subject 2000* shows a relatively high degree of variation in expression with oxygen tension, while *subject 1973* shows a relatively low degree of variation. Table 2 reports the mean values obtained for the slope and intercept (at 10% O_2) for each subject for each gene.

The results of ANOVA on the gradients of the responses are shown in Table 3. First, the interaction of "subject" with

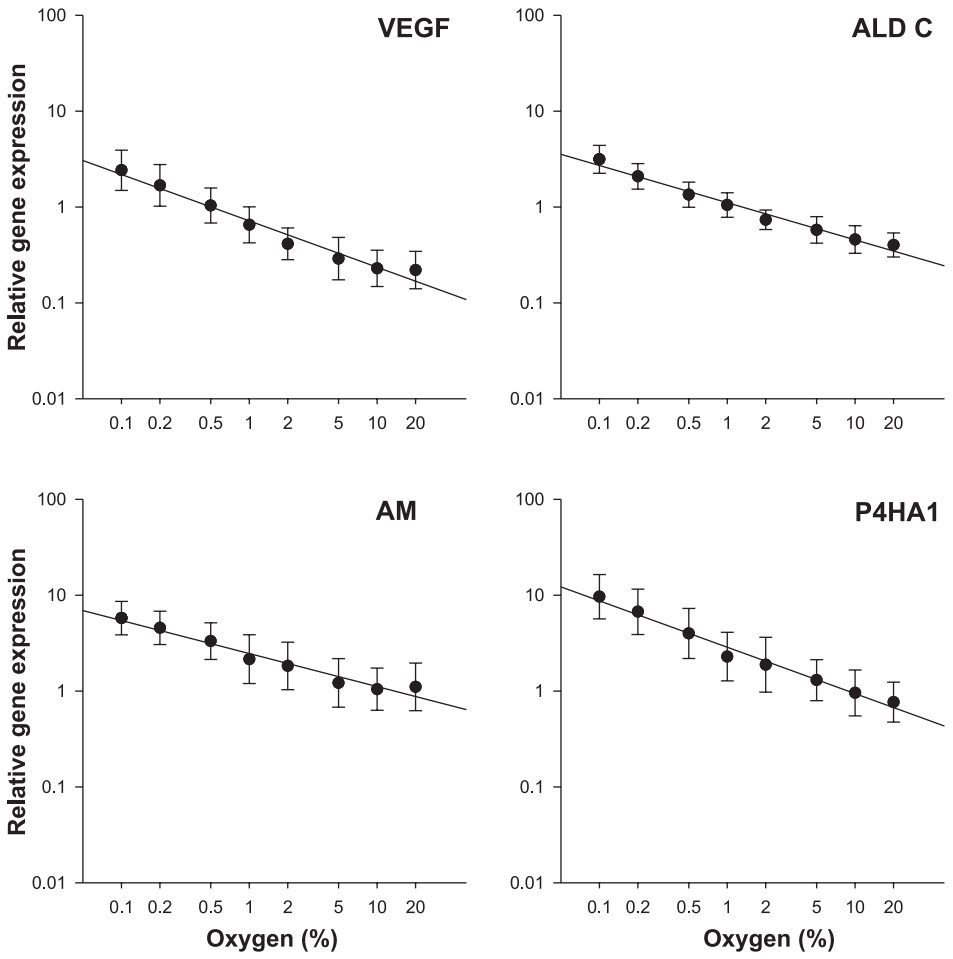

Fig. 3. Mean values for relative expression of *VEGF*, *ALDC*, *AM*, and *P4HA1* at 20–0.1% O_2 from all repeat experiments on all subjects plotted on a double logarithmic scale. Error bars are ±SD for the intersubject variability. Lines are the best fit by simple linear regression.

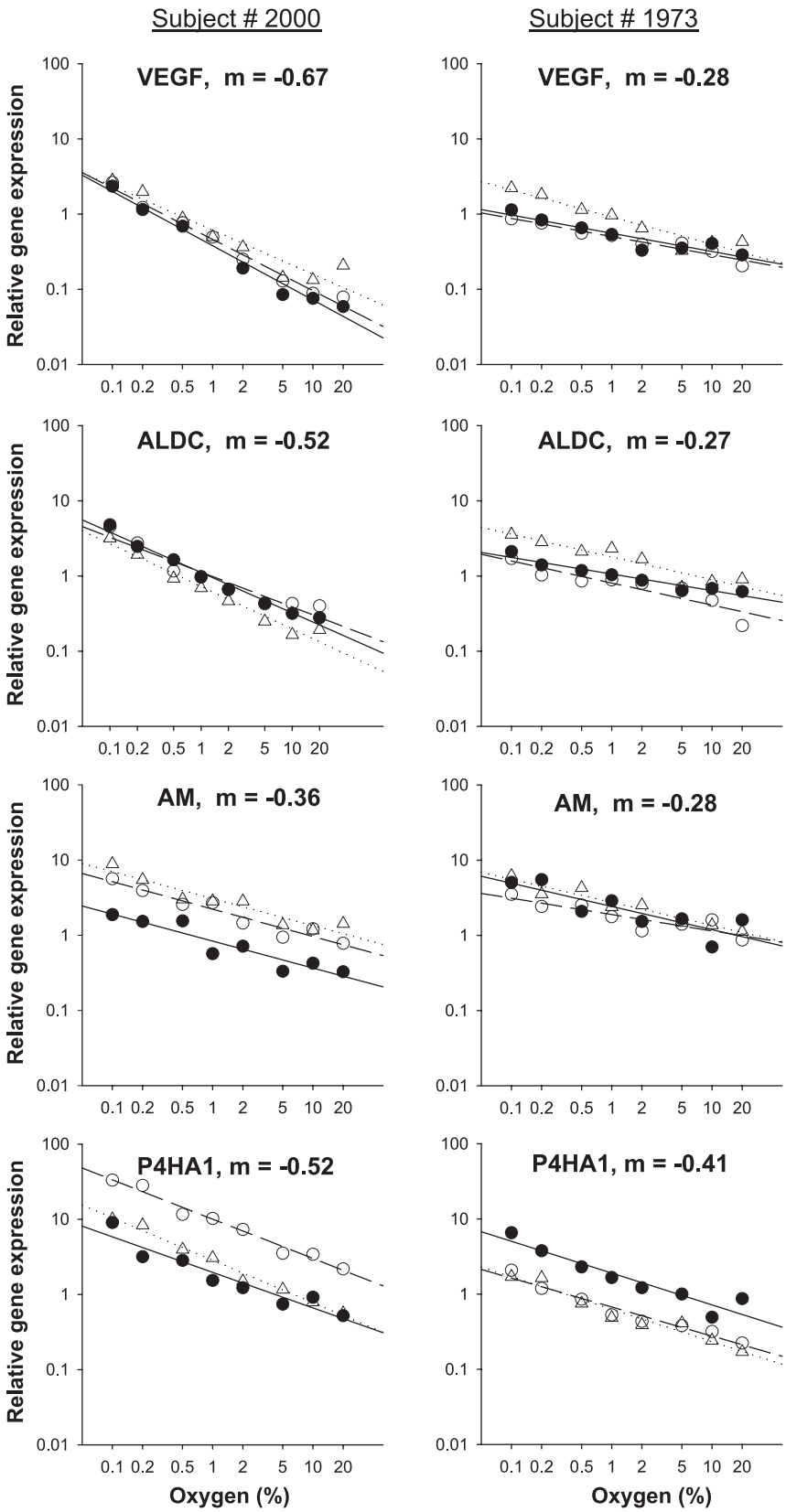

Fig. 4. Relative values of *VEGF*, *ALDC*, *AM*, and *P4HA1* expression over a range of oxygen tensions in 3 repeat experiments performed on peripheral blood lymphocytes of *subject 2000*, showing a high sensitivity to changing oxygen tensions, and of *subject 1973*, showing, in contrast, a low sensitivity to changing oxygen tensions. Mean values for the gradients of the responses for each transcript (m) are shown.

Table 2. *Gradients and intercepts (log values at 10% O_2) for log-log relationships between gene expression and percent oxygen concentration*

	Gradients				Intercepts			
Subject No.	*VEGF*	*ALDC*	*AM*	*P4HA1*	*VEGF*	*ALDC*	*AM*	*P4HA1*
1096	−0.43	−0.31	−0.43	−0.47	−0.72	−0.25	−0.19	0.05
1229	−0.5	−0.35	−0.39	−0.52	−0.86	−0.48	−0.07	−0.34
1286	−0.57	−0.36	−0.37	−0.55	−0.68	−0.18	−0.28	−0.16
1490	−0.41	−0.37	−0.37	−0.52	−0.57	−0.3	−0.08	−0.11
1506	−0.59	−0.52	−0.36	−0.49	−0.6	−0.48	0.2	0.28
1508	−0.57	−0.45	−0.33	−0.42	−0.36	−0.21	0.37	0.08
1513	−0.44	−0.36	−0.31	−0.48	−0.53	−0.38	0.29	0.16
1522	−0.4	−0.37	−0.26	−0.46	−0.47	−0.39	0.31	0.12
1973	−0.28	−0.27	−0.28	−0.41	−0.48	−0.21	0.09	−0.45
2000	−0.67	−0.52	−0.36	−0.52	−0.98	−0.53	−0.11	0.06
Mean	−0.49	−0.39	−0.35	−0.48	−0.63	−0.34	0.05	−0.03
SD	0.12	0.08	0.05	0.05	0.19	0.13	0.23	0.23

VEGF, vascular endothelial growth factor; *ALDC*, aldolase C; *AM*, adrenomedullin; *P4HA1*, prolyl-4-hydroxylase α1.

"experimental repeat" reflects the variability between experiments (i.e., between data derived from different blood samples) that is common across the four genes studied. This did not quite reach statistical significance ($P = 0.057$), which suggests that the data are not overly affected by variation between experiments. Second, the interaction of "gene" with "subject" (gene*subject) reflects variation between subjects that is different between different genes. This is significant ($P < 0.001$), but because this variation is not common across the four HIF-regulated genes studied, it cannot be ascribed to differences within the HIF system. Finally, the "subject" term reflects the variation in induction by hypoxia between subjects that is common across all four genes studied. This is significant ($P = 0.008$) and forms the principal finding of the study. Effectively, the result demonstrates that subjects can be phenotyped according to their overall sensitivity to the induction of oxygen-regulated gene expression by hypoxia. The factor of "gene" in the ANOVA is of no interest because genes are expected to vary naturally in their degree of induction by hypoxia and, in this analysis, it is also confounded by the scaling process.

The results of the ANOVA of the intercepts at 10% O_2 are shown in Table 4. First, the interaction of "subject" with "experimental repeat" is not significant ($P = 0.123$), suggesting that the data for the intercepts are also not overly affected by variation between experiments. Second, the interaction of "gene" with "subject" (gene*subject) is significant ($P = 0.001$), suggesting that there is variation between subjects that is different between different genes. Finally, in contrast to the data for the gradients, the "subject" term is not significant ($P = 0.115$), suggesting that any variation in the intercept values among the subjects is unlikely to be due to overall differences in the HIF system.

As a final analysis, the ANOVA was repeated on the values for gradient and intercept before they were scaled so as to have equal variance for each gene. In no case did the conclusions from this analysis differ from those obtained from the scaled values.

DISCUSSION

The results from this study demonstrated that there were significant differences between individuals for the basal expression of the various HIF-regulated genes when no hypoxic stimulus was present. For these basal expression levels, there was no common pattern across the genes studied, suggesting

Table 3. *ANOVA on scaled gradients for double logarithmic relationship between gene expression and oxygen tension*

Source	Sum of Squares	Degrees of Freedom	Mean Square	*F* Value	*P* Value
Intercept					
Hypothesis	3,593.701	1	3,593.701	723.373	0.000
Error	44.712	9	4.968*		
Gene					
Hypothesis	465.756	3	155.252	127.026	0.000
Error	32.999	27	1.222†		
Subject					
Hypothesis	44.712	9	4.968	3.289	0.008
Error	41.919	27.751	1.511‡		
Gene * subject					
Hypothesis	32.999	27	1.222	3.006	0.000
Error	24.391	60	0.407§		
Experimental repeat * subject					
Hypothesis	13.897	20	0.695	1.709	0.057
Error	24.391	60	0.407§		

Each data point in the analysis consists of 1 gradient for 1 gene from 1 experiment; this gives a total of 120 data points. The scaling ensured that the variance for the measurements for each gene was unity. The factors employed are as described in MATERIALS AND METHODS. ANOVA, analysis of variance; MS, mean square. *MS(subject); †MS(gene * subject); ‡MS(gene * subject) + MS(experimental repeat * subject) − MS(error); §MS(error).

Table 4. *ANOVA on scaled intercepts at 10% O_2 for double logarithmic relationship between gene expression and oxygen tension*

Source	Sum of Squares	Degrees of Freedom	Mean Square	*F* Value	*P* Value
Intercept					
Hypothesis	158.576	1	158.576	50.948	0.000
Error	28.012	9	3.112*		
Subject					
Hypothesis	28.012	9	3.112	1.812	0.115
Error	43.765	25.475	1.718†		
Gene					
Hypothesis	171.103	3	57.034	39.127	0.000
Error	39.357	27	1.458‡		
Gene * subject					
Hypothesis	39.357	27	1.458	2.685	0.001
Error	32.568	60	0.543§		
Experimental repeat * subject					
Hypothesis	16.062	20	0.803	1.480	0.123
Error	32.568	60	0.543§		

Each data point in the analysis consists of 1 intercept for 1 gene from 1 experiment; this gives a total of 120 data points. The scaling ensured that the variance for the measurements for each gene was unity. The factors employed are as described in MATERIALS AND METHODS. *MS(subject); †MS(gene * subject) + MS(experimental repeat * subject) − MS(error); ‡MS(gene * subject); §MS(error).

that different regulatory mechanisms are important in determining basal expression for the different genes. However, there were also significant differences between individuals in the degree to which expression of these genes was induced by hypoxia. In contrast with levels of basal expression, there was a common pattern across the genes for the induction in expression by hypoxia, such that if one gene was strongly induced by hypoxia in an individual then this would tend to be the case for all the other genes within that individual. It is this result that suggests that individuals differ significantly in a factor that is common to all the genes studied in relation to the induction of their expression by hypoxia.

As outlined in the introduction, it is already recognized that individuals differ in many of their phenotypic responses to hypoxia. Generally, these phenotypes are very complex and integrative in nature, and their origins remain obscure. In contrast, the phenotype of this study is much narrower and is tightly linked to one particular biochemical pathway—the HIF transcription activation pathway. It was therefore less likely that significant variation would exist between individuals, but because it does exist, it is suggestive of the presence of functional genetic variants in humans within the HIF transcription activation pathway. Although not investigated in this study, the phenotype provides a tool to explore the genetic origins of functional variation within the HIF pathway and to explore associations with more complex phenotypes.

There have been relatively few other human studies of variation in the transcription of HIF-regulated genes. However, variation in the hypoxic induction of *VEGF* has been investigated in peripheral blood monocytes (PBM) taken from patients with coronary artery disease (43, 49) and also from patients with diabetic retinopathy (31). Positive associations were observed between the level of induction of *VEGF* by hypoxia and both the degree of coronary artery collateral formation and the degree of diabetic retinopathy. The authors of these studies suggest that the variability in induction of *VEGF* by hypoxia may reflect interindividual differences in the responsiveness of the HIF system to hypoxia (43). However, other explanations remain possible. First, because only the expression of *VEGF* was investigated, it is possible that the regulatory differences were entirely specific to VEGF and did not involve the HIF signaling pathway. Second, in these experiments the PBM were resuspended in the patients' own plasma before incubation under euoxic or hypoxic conditions (43), and it is therefore possible that differences in the plasma constituents could underlie some of the variability in induction of VEGF by hypoxia. The present study controls for both these issues, and thus provides further support for the notion that the sensitivity of VEGF to induction by hypoxia is related to the sensitivity of other HIF-regulated genes to hypoxia.

One potential problem with our experimental system is that the method used to isolate PBL was not selective for lymphocyte subtypes. Although such cell types are closely related, there is still a theoretical possibility that the differences seen in the responses reflect, to some extent, differences in the proportions of lymphocyte subtypes among the subjects. Against this possibility we have found that, for *ALDC*, the average gradient of the log-log relationships between gene expression and oxygen tension for Epstein-Barr virus-transformed B lymphocytes from a number of normal subjects was very similar to that of the mixed population of PBL in this study (J. T. S. Brooks, unpublished observations).

As noted in the introduction, HIF plays an important role in promoting angiogenesis, both in cardiovascular disease and cancer—the latter supporting the development of metastases (6, 13, 43, 45). There is also evidence that the degree of induction of HIF-regulated genes in human cancers is a powerful predictor of outcome, with higher degrees of induction associated with poorer prognosis (15, 53). Our study raises the possibility that individuals may differ innately in this process. Although this study was not primarily concerned with the roles of the HIF system in PBL, it is nevertheless worth noting its involvement in a number of different functions. These include its involvement in autoimmunity (29), in myeloid cell function in inflammation (18), in T-lymphocyte physiology (11, 16, 30, 33), and in fighting bacterial, viral, and parasitic infection (56).

One interesting area of speculation concerns the particular genetic variation that gives rise to the variation in the sensitivity of the HIF system to decreasing oxygen tension. The major regulatory step in this system is the oxygen-dependent

hydroxylation of HIF at specific proline residues that tags the protein for subsequent von Hippel-Lindau (VHL) protein binding and subsequent ubiquitin-mediated proteasomal degradation (25, 26). The recent identification of a mutation in the HIF-2α gene that is associated with congenital erythrocytosis suggests that variation within the HIF genes themselves could be one source of variation (37). The prolyl hydroxylation of HIF is mediated by three isoenzymes termed prolyl hydroxylase domain (PHD) proteins (19), the activities of which are dependent on oxygen as a cosubstrate together with Fe^{2+} and ascorbate as cofactors. The three PHD isoenzymes remain active at oxygen tensions at least as low as 0.2% (4), and the rate of hydroxylation is exquisitely sensitive to oxygen tension over the range of values typically encountered in mammalian cells (24). Thus genetic variability within any of the genes coding for the PHD isoenzymes and/or any factors that influence the activity of the PHD enzymes may well be a source of interindividual differences in the sensitivity of the HIF system. Recently, mutations within the PHD2 gene have been identified that are associated with a congenital polycythemia syndrome characterized by inappropriately raised Epo levels (35, 36). In vitro transfection studies suggest that these PHD2 variants have reduced hydroxylase activity that results in raised levels of transcription of HIF-regulated genes compared with wild-type PHD2 (35, 36).

In conclusion, we have demonstrated that it is possible to resolve differences between normal individuals in the induction by hypoxia of a range of HIF-regulated genes. The use of PBL to derive this phenotype provides an approach that is both reproducible and relatively noninvasive, in the sense that it does not require tissue biopsy of specific cell types. As such, our experimental system provides an intermediate biochemical phenotype by which the cellular responsiveness to hypoxia may be quantified for individuals. Such a system provides an opportunity to try to relate variations in the HIF system both upward toward the variations in integrated responses to hypoxia observed between individuals and downward toward genetic variations within the HIF system that may underlie the biochemical phenotype.

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GRANTS

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