Circulating Fetal DNA in Maternal Plasma Is Increased in Pregnancies at High Altitude and Is Further Enhanced by Preeclampsia, Xiao Yan Zhang,1 Yiming Wang,2 Suqin Chen,2 Labu,3 Pabuzhuoma,3 Gesangzhougab,2 Ou Zhuzhuangmu,4 Xiaoying Pan,5 Ninghu Zhu,5 Cornelia Hahn,1 Berthold Huppertz,6 Wolfgang Holzgreve,7,8 and Stimu Hahn†† (1 University Women’s Hospital/Department of Research, University of Basel, Basel, Switzerland; 2 Department of Medical Genetics, Sun Yat-Sen University, Guangzhou, Guangdong Province, and Lhasa, Tibet. Written informed consent was obtained before donation of an 8-mL venous blood sample. All samples were stored frozen until use. Plasma samples from Lhasa were air-freighted frozen to Guangzhou for analysis. Previous studies have shown that the shipping of maternal plasma samples under conditions similar to those we used for this study does not lead to significant differences in circulating fetal DNA concentrations compared with samples processed locally (7, 10, 11). All plasma samples were analyzed under identical conditions in parallel at the Division of Medical Genetics, Sun-Yat University, Guangzhou, China, by real-time PCR as described previously (7). Strict anticontamination procedures were used throughout, and no false-positive results were recorded. The data acquired in Guangzhou were analyzed at the University of Basel (Basel, Switzerland) by use of SPSS® and Microsoft Excel® software packages. Significance for these nonparametric data were determined by the Mann–Whitney test.

We examined circulating fetal DNA concentrations in Han Chinese (n = 19) with uncomplicated pregnancies at sea level in comparison with recently migrant Han Chinese (n = 21) as well as ethnic Tibetans (n = 27) living in Lhasa (altitude = 3650 m; Table 1). We also examined the possible influence of preeclampsia at high altitude on circulating fetal DNA concentrations in both Han Chinese (n = 11) and ethnic Tibetans (n = 15; Table 1). Preeclampsia...
sia was determined by a blood pressure of ≥140/90 mmHg in two determinations 4 h apart or by a diastolic blood pressure of ≥110 mmHg and an associated proteinuria of ≥300 mg/48 h after 20 weeks of gestation in previously normotensive women, as described previously (7).

Our analysis indicated that concentrations of circulating fetal DNA were significantly increased by a factor of almost 4 in the high-altitude pregnancies compared with the control cohort at sea level (Table 1 and Fig. 1A). For women with noncomplicated pregnancies, we observed no significant difference between ethnic Tibetans and migrant Han Chinese living in Lhasa with regard to circulating fetal DNA concentrations (Table 1 and Fig. 1A) or fetal birth weight (Table 1). In those pregnancies at high altitude that were affected by preeclampsia, we observed an additional significant increase in circulating fetal DNA in both population groups (Table 1 and Fig. 1B). Again, we found no significant difference in fetal DNA concentrations between these two groups. What is clear, however, is that even under these conditions at high altitude, preeclampsia is associated with an increment in circulating fetal DNA concentrations, in a manner similar to what has been reported previously in studies conducted at lower altitudes (6, 7).

Our findings are of interest because they indicate that the placental changes that occur at high altitude are mirrored in the release of cell-free DNA by these tissues,

### Table 1. Characteristics of high-altitude pregnancies compared with those at sea level.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gestational age, weeks</th>
<th>BP, a mmHg</th>
<th>Fetal mass, g</th>
<th>Fetal DNA, GE/mL of maternal plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Han Chinese at sea level</td>
<td>19</td>
<td>Median: 39.1</td>
<td>106/68</td>
<td>3350</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range: 38.3–41.2</td>
<td>98–130/56–80</td>
<td>2570–4250</td>
<td>5.5–87.5</td>
</tr>
<tr>
<td>Lhasa Han Chinese</td>
<td>21</td>
<td>Median: 39.6</td>
<td>100/60</td>
<td>3200</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range: 35–41</td>
<td>80–120/60–86</td>
<td>2285–3800</td>
<td>17.5–212.5</td>
</tr>
<tr>
<td>Tibetans</td>
<td>27</td>
<td>Median: 39</td>
<td>100/70</td>
<td>3200</td>
<td>76.5</td>
</tr>
<tr>
<td>Han Chinese with preeclampsia</td>
<td>15</td>
<td>Median: 39</td>
<td>150/105</td>
<td>2800</td>
<td>810</td>
</tr>
<tr>
<td>Tibetans with preeclampsia</td>
<td>11</td>
<td>Median: 39</td>
<td>140/90</td>
<td>3200</td>
<td>859.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range: 38–39</td>
<td>120–170/90–120</td>
<td>2100–3900</td>
<td>50.3–8547.5</td>
</tr>
</tbody>
</table>

*a* BP, blood pressure; GE, genome-equivalents.

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![Fig. 1. Box-plots of circulating fetal DNA concentrations in noncomplicated pregnancies at sea level (Guangzhou) and high altitude (Lhasa; 3650 m; A), and those affected by preeclampsia (PET) at high altitude (B).](image-url)

**Fig. 1.** Box-plots of circulating fetal DNA concentrations in noncomplicated pregnancies at sea level (Guangzhou) and high altitude (Lhasa; 3650 m; A), and those affected by preeclampsia (PET) at high altitude (B). **Thick black lines**, medians; **boxes**, 25th and 75th percentiles; **whiskers**, 10th and 90th percentiles. Note that the scales of the y axes differ between panels A and B. Concentrations are given in genome-equivalents/mL of maternal plasma.
concurrent with the attempt to adapt to and overcome the lowered oxygen tension. Of further interest is that no significant difference in circulating fetal DNA concentrations was discernable between ethnic Tibetans and recent migrant Han Chinese. This finding is rather unexpected because previous studies have supported the hypothesis that ethnic populations, such as Tibetans who have lived for almost 5 millennia at high altitude, have adapted to low oxygen concentrations and consequently have better pregnancy outcomes than do newly arrived migrant populations (16). We did not observe any influence of ethnicity concerning the release of fetal DNA, which suggests that the underlying placental alterations are similar in both groups. What does seem likely, however, is that residents who have lived for extended periods at high altitudes have developed optimal strategies for adapting their placental tissues to the underlying deficiencies to ensure an optimal pregnancy outcome (16). This may be reflected in the increased occurrence of intrauterine growth restriction in Han Chinese at high altitude, in contrast to native Tibetans, a trend we also observed in our study. i.e., babies born to Han Chinese women living at high altitude were smaller than those born to Han Chinese women living at sea level. We also observed some evidence of a further reduction in fetal weight for babies born to Han Chinese women with preeclampsia at high altitude, whereas no such difference was apparent in comparable Tibetan study groups. These findings will, however, need to be confirmed in larger studies.

In summary, analysis of circulating fetal DNA may not only be useful for the noninvasive prenatal assessment of fetal genetic traits; in the future, it may also be a unique tool for the study of anomalous placentation. In this context it will be interesting to determine whether circulating fetal DNA concentrations behave in a similar manner in pregnancies at high altitude.

References

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Genotyping of Hepatitis C Virus by Melting Curve Analysis: Analytical Characteristics and Performance, Doris M. Haverstick, Grant C. Bullock, and David E. Bruns (Department of Pathology, University of Virginia, Charlottesville, VA; * address correspondence to this author at: Department of Pathology, University of Virginia Medical School, PO Box 800214, Charlottesville, VA 22908-0214; fax 434-924-8060, e-mail dmh2t@virginia.edu)

Knowledge of the hepatitis C virus (HCV) genotype is important in guiding antiviral therapy (1). Viral pharmacogenomic studies have demonstrated that patients infected with genotype-1 HCV respond poorly to interferon-ribavirin therapy and may require a longer course of therapy (1–3). In the United States, where the most common HCV types are 1a/b, 2a/c, 2b, and 3a, it is particularly important to distinguish patients infected with type 1a/b from those infected with types 2 and 3. To meet the need for this information, we have developed a rapid-cycle, real-time PCR assay with melting-curve analysis for genotyping of HCV (4). This method uses reverse transcription-PCR performed in a block cycler followed by a seminested PCR with product identification using fluorescence resonance energy transfer (FRET) probes and DNA melting curves in a single tube. The FRET probes were designed to identify HCV types 1, 2a/c, 2b, 3a, and 3b/4. Other less common genotypes will likely either not be amplified (types 6b, 7b, and 11a) or will produce a product with a non-type 1 melting temperature (Tm) (4).

Real-time PCR has gained widespread use in clinical analyses since its introduction in 1991 (5), but little has been published on the performance characteristics of such assays over periods longer than a few days or weeks. The objective of the present study was to determine the analytical characteristics of the above HCV genotyping assay and its performance in routine use. The study period covered 23 months with 92 runs performed by six operators on four different LightCycler® instruments, using the exact assay described above (4).

Patient samples were analyzed in groups of 5 to 13. Each analytical run also contained three quality-control