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SHORT REPORT

Precision medicine in diabetes: A non-invasive prenatal diagnostic test for the determination of fetal glucokinase mutations

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ABSTRACT

Hyperglycemia caused by mutations in the glucokinase gene, *GCK*, is the most common form of monogenic diabetes. Prenatal diagnosis is important, as it impacts on treatment. This study reports a monogenic non-invasive prenatal diagnostic (NIPD-M) test on cell-free DNA in maternal plasma using the relative haplotype dosage. In three pregnancies of two families with known maternal *GCK* mutations, the fetal genotype was determined unambiguously already at 12 weeks of gestation. In summary, proof is provided of the feasibility for NIPD-M in *GCK* diabetes.

INTRODUCTION

Monogenic diabetes comprises a diverse group of metabolic disorders caused by defects in single genes[1,2] *GCK* diabetes is the cause of gestational diabetes in 1–2% of affected women[3,4] The fetal *GCK* genotype impacts on treatment recommendations, which differ from other causes of gestational diabetes[5] Non-affected fetuses are prone to excessive weight gain due to the hyperglycemic maternal environment and are exposed to the risk of macrosomia and related complications[5–7] By contrast, an affected fetus will present with a normal weight gain, because of a higher threshold to elicit adequate insulin secretion. Maternal insulin treatment is thus only recommended in the presence of fetal signs for macrosomia. Here we apply 'relative haplotype dosage' (RHDO) analysis to non-invasive prenatal diagnostic (NIPD) of *GCK* mutations at distinct time points during pregnancy. The method relies on allelic imbalance caused by small amounts of fetal circulating cell-free DNA (ccfDNA) in maternal ccfDNA. The allelic balance is 50:50 in a heterozygous mother carrying a heterozygous fetus, but becomes skewed if the fetus is homozygous wild-type (Figure 1). The low abundance of circulating DNA makes it difficult to achieve significance by testing only the mutation, therefore RHDO queries adjacent single nucleotide polymorphisms (SNPs) to increase the statistical power.

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RESEARCH DESIGN AND METHODS

The study was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from both parents.

A panel of 98 SNPs spread over 0.7 Mb around the *GCK* gene was designed, additionally including the p. Gly246Arg mutation (Figure 2). A molecular barcode library was built to sequence 50-nucleotide regions encompassing each SNP. Parental haplotypes were reconstructed by sequencing whole blood DNA from the parents and their first child (Tables S1–S3). The same library was used to sequence ccfDNA extracted from maternal plasma and controlled for quality as described[8] The maternal haplotype inherited by the fetus was determined by RHDO. See Methods S1 for details.

RESULTS

In family A, the mother was diagnosed with *GCK* diabetes at the age of 14, with a HbA1c of 42 mmol/mol (6%). She carried a heterozygous *GCK*:c.736G>A, p. Gly246Arg mutation. At 12 and 30 weeks of gestation, ccfDNA was extracted from maternal blood. A baby girl was born at 38 6/7 weeks with a birth weight of 3.24 kg (p25-50). DNA was extracted from cord

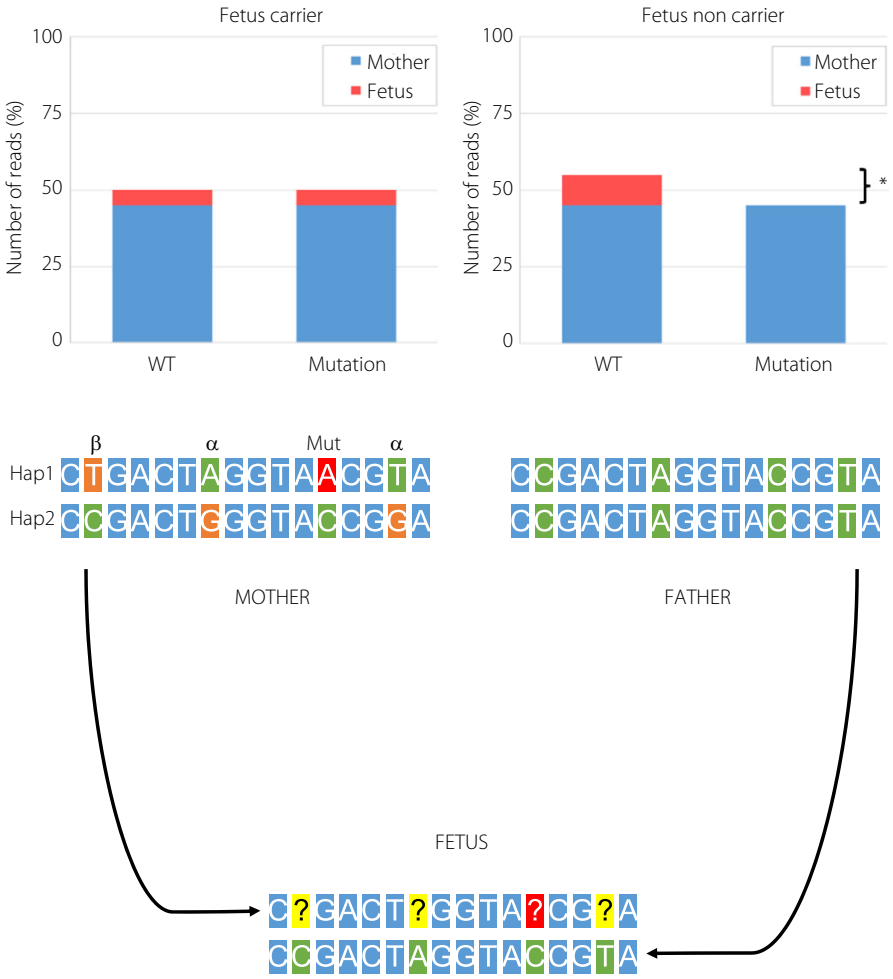

Figure 1 | Principle of relative haplotype dosage analysis. Top panel: Fetal genotype is inferred from the WT/mutant ratio in sequence reads (which is 0.5 in maternal DNA, as the mother is heterozygous). If the fetus does not carry the mutation, there is an excess of WT reads (*), equivalent to the fraction of fetal DNA. Bottom panel: To increase sensitivity and statistical significance, the analysis is extended to a panel of SNPs on either side of the mutation, chosen for their high rate of heterozygosity in the general population (cartoon is not to scale: SNPs are actually 5 to 10 kb apart). Informative SNPs, i.e. those for which the mother is heterozygous and the father is homozygous, are subdivided into alpha and beta SNPs, depending whether the paternal allele corresponds to maternal haplotype Hap1 (high-risk) or Hap2 (low-risk).

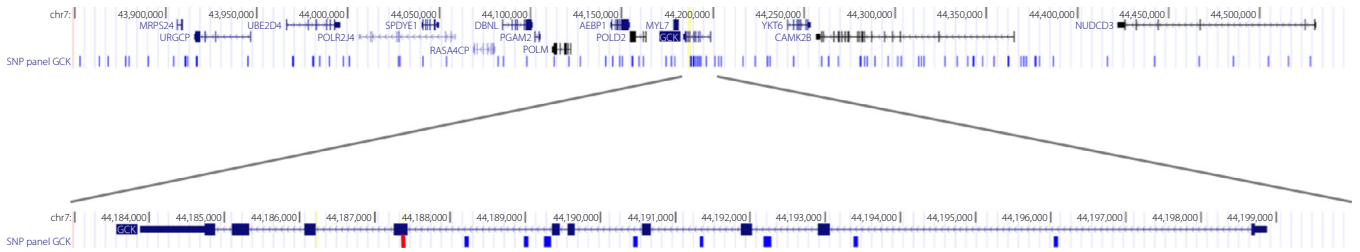

Figure 2 | SNP panel for *GCK*. Top panel: Genomic region [hg19] chr7:43,850,000-44,550,000, centered on the *GCK* gene. Bottom panel: zoom on the *GCK* gene region, [hg19] chr7:44,183,000-44,200,000. In each panel, the top track contains genomic coordinates, the middle track displays gene content according to GENCODE v32 and the bottom track shows the regions sequenced, each encompassing a SNP. The location of the maternal mutation *GCK*:p. Gly246Arg is marked in red. Family B's mutation *GCK*:p. Asp278Glu was not part of the panel. Figure produced with the UCSC genome browser: https://genome.ucsc.edu. GENCODE track is from: Harrow *et al.*[18].

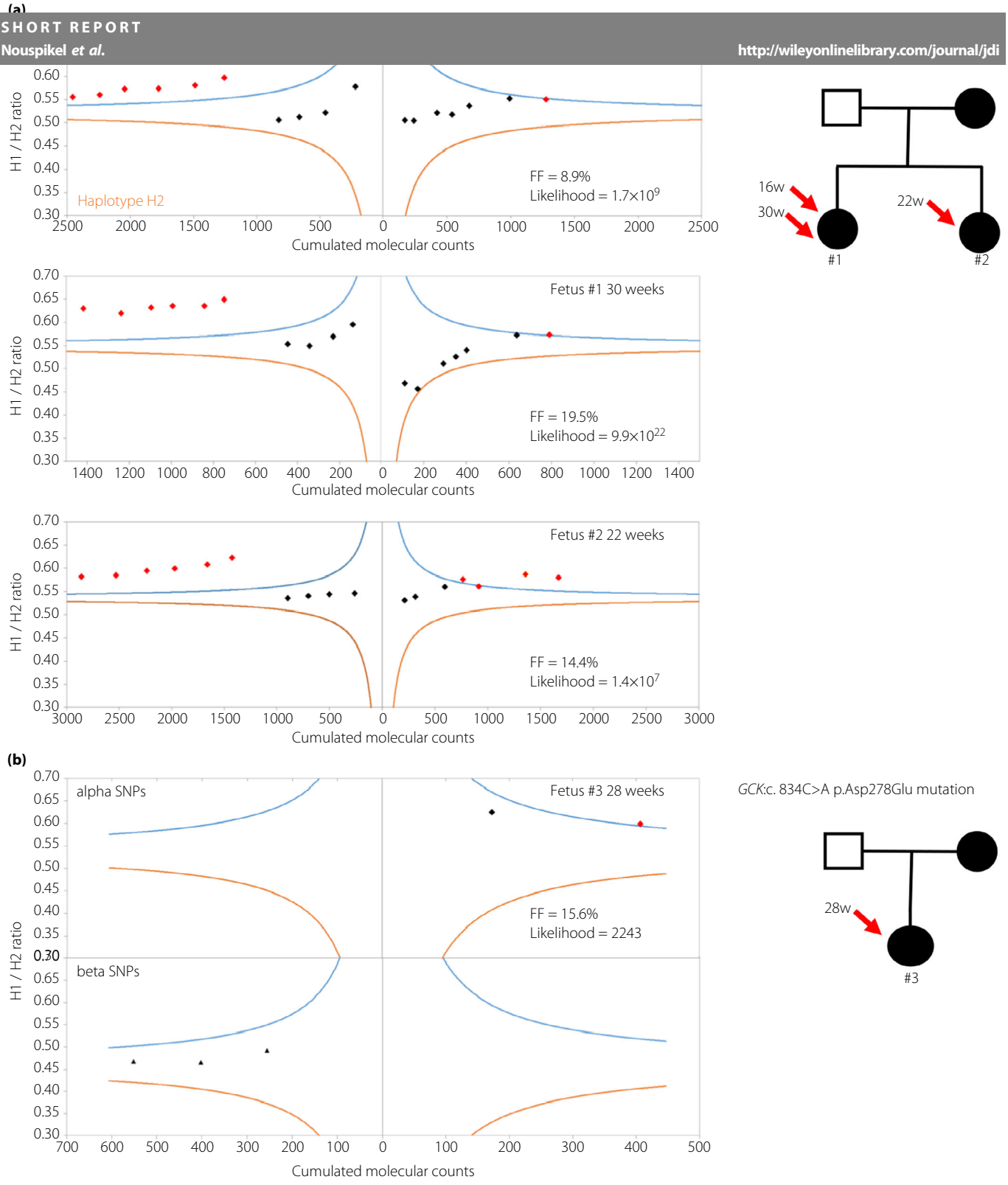

Figure 3 | Relative haplotype dosage analysis in the *GCK* region. (a) Family tree and RHDO analysis for family A. Fetus 1 12-week gestation and 30-week gestation, fetal DNA fraction (FF) = 8.9% and FF = 19.5%, respectively. Fetus 2, 22-week gestation, FF = 14.4%. (b) Family tree and RHDO analysis for family B. Fetus 3 28-week gestation with FF = 15.6%. Each panel is centered on the position of the maternal mutation, diamond symbols represent SNPs on either side of the mutation. The *x*-axis is the cumulated number of analyzed molecules for all SNPs so far, the *y*-axis is the allelic ratio of haplotype 1 over haplotype 2. Diagnostic thresholds for haplotype 1 (high-risk, blue line) and 2 (low-risk, orange line) are calculated from the fetal fraction with a likelihood ratio of 1200. Red diamonds indicate a significant result. Informative SNPs, i.e. those for which the mother is heterozygous and the father is homozygous, are subdivided into alpha and beta SNPs, depending on whether the paternal allele corresponds to maternal haplotype Hap1 or Hap2. Beta SNPs are not shown for family A.

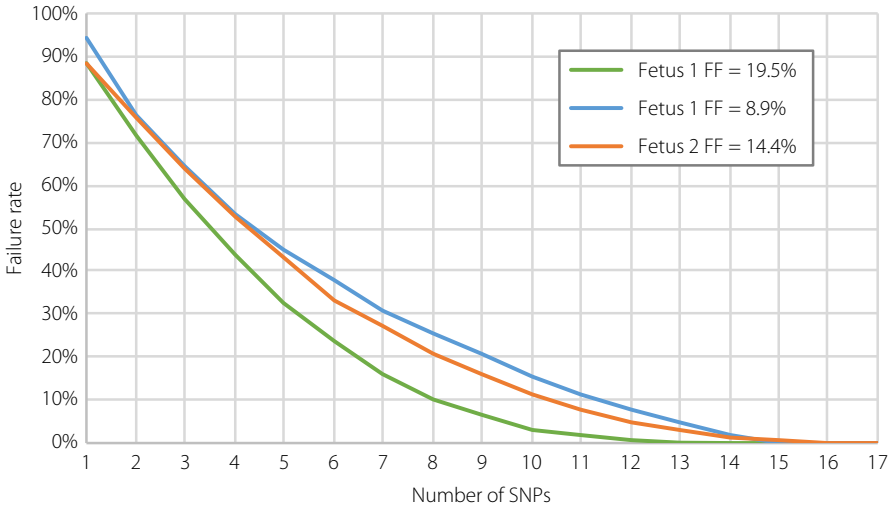

Figure 4 | Effect of the number of available SNPs. The three samples of family A were analyzed as above, using only randomly chosen subsets of the 17 informative SNPs available. A maximum of 10,000 combinations were tested for each number of SNPs and the fraction of tests failing to reach a likelihood threshold of 1200:1 is displayed.

blood to validate the NIPD results and to serve as a surrogate 'previous child' for the determination of parental haplotypes. During the second pregnancy, ccfDNA was extracted at 22 weeks. A baby girl was born at 37 6/7 weeks with a birth weight of 3.045 kg (P50) (Figure 3). Since the test was in the validation phase, the results were not used for clinical decisions; but no insulin treatment was required.

In family B, the mother carried a heterozygous *GCK* c.834C>A, p. Asp278Glu mutation, and ccfDNA was extracted at 28 weeks of gestation. A baby girl was born at 37 3/7 weeks with a normal birth weight (2.62 kg, P10-P25) and her DNA was used for parental haplotype determination. Since the fetal genotype was only confirmed after birth, the mother was treated with insulin analogs at mealtimes.

The analyses were performed at different timepoints to test several fetal DNA fractions (FF), which increase in maternal plasma with the advancement of pregnancy. The FF was determined with SNPs for which the parents were homozygous for different alleles (Figure 3). For the first pregnancy of family A, FF was 8.9% at 12 weeks and 19.5% at 30 weeks; for the second pregnancy it was 14.4% at 22 weeks. For family B, the FF was 15.6% at 28 weeks. In all cases, RHDO unambiguously demonstrated that the fetus had inherited the high-risk maternal haplotype, i.e. the one with the familial mutation (Figure 3). Due to the low number of informative SNPs for family B (only 5, vs 17 for family A), we had to repeat the analysis with a larger quantity of DNA to reach the diagnostic threshold of 1200 (i.e. haplotype 1 is at least 1200 times more likely than haplotype 2). All results were confirmed by DNA analysis of the children.

We then investigated the effect of the number of informative SNPs on the likelihood of experimental failure. We reanalyzed data from family A using randomly selected subsets of the available 17 informative SNPs. We tested a maximum of 10,000 combinations for any number of SNPs and asked what fraction of these failed to reach the 1200:1 likelihood threshold. Figure 4 demonstrates that, depending on FF, a minimum of 12 to 14 SNPs suffices to virtually exclude failure. Statistically, in a panel of 100 SNPs, 25 are expected to be informative. However, since these SNPs are in linkage disequilibrium by design, it is possible that a lower (or higher) number is available, as in the extreme situation of family B.

DISCUSSION

Analysis of ccfDNA fragments in maternal plasma is the basis of non-invasive prenatal tests[9] Fetal ccfDNA, released during apoptosis of trophoblastic cells, represents a minor fraction (5–20%) of the total ccfDNA, the remainder being of maternal origin. Several NIPD tests for monogenic disorders such as Duchenne muscular dystrophy, spinal muscular atrophy, β-thalassemia, and congenital adrenal hyperplasia have been developed (reviewed in[10]). Our non-invasive test relies on high throughput sequencing and is thus significantly more expensive than most invasive tests. But the latter, associated with the risk of fetal loss, are in any case difficult to justify given the mild nature of *GCK* diabetes. Other non-invasive methods, collectively known as relative mutation dosage (RMD), have been described, which successfully tested for the sole presence of the mutation[11,12] RMD is hampered by the low abundance of circulating DNA, which makes it difficult, although feasible, to achieve statistical significance by testing a single genomic position. Additionally, relative mutation dosage in itself does not allow us to determine FF, which is critical to calculate significance. Relative haplotype dosage overcomes these limitations by

using SNPs but requires genotyping a prior offspring of the same couple. When such a sample is not available, an alternative is genotyping the maternal grand-parents. As a last resort, techniques such as long reads sequencing, targeted locus amplification phasing[13] or barcoded gel beads technology could be envisioned. If the targeting panel does not yield informative SNPs (e.g. with consanguineous parents[10]) the method could be improved by designing a larger panel, containing more closely spaced SNPs and/or spanning a larger region. Alternatively, drawing more blood would allow us to build libraries from higher amounts of DNA, achieving higher molecular counts and thus greater significance with the same number of SNPs.

There are no evidence-based guidelines for the clinical management of *GCK* diabetes during pregnancy[4,14,15] In current practice, starting at 26 weeks of gestation, fetal growth is assessed every 14 days[4] Fetal abdominal circumference rising above the 75th percentile is considered to be an indirect sign of macrosomia development and thus of fetal *GCK* non-carrier status and maternal insulin treatment is usually started[16] In affected newborns, intensive maternal insulin treatment leads to a decreased fetal insulin secretion and hence to a reduction in birth weight[7,17] In addition, there is a high incidence (23%) of severe maternal hypoglycemia[17] This test should be an invaluable help in deciding on maternal treatment during pregnancy. Most notably, by simply designing new SNP panels, relative haplotype dosage could be adapted to other monogenic diabetes genes, introducing precision medicine during pregnancy.

ACKNOWLEDGMENTS

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DISCLOSURE

The authors declare no conflict of interest.
Approval of the research protocol was obtained from the Ethics Committee of the Canton of Geneva, Switzerland.
Informed consent was obtained from all participants or their legal representatives.
The approval date of Registry and the Registration No. of the study/trial was March 28, 2018, Registration No: PB_2018-00092 (11-140).
Animal Studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Haplotypes for family A
Table S2 | Haplotypes for family B
Table S3 | Sequencing parameters
Methods S1 | Panel design, library construction, DNA sequencing and analysis