Whereas 984 incident cases of US-based NAFLD developed during 13,882.4 person-years of follow-up (incidence density (ID), 74.1 per 1000 person-years; 95% CI, 69.5–78.7], 700 incident cases of increased ALT, de-

To the Editor:

Programs to screen newborns for congenital disorders are based on analysis of dried blood spot samples (DBSS), which have been shown to be robust and convenient for collection, transport, and storage. Because blood samples are collected with no selection, and coverage of the population is essentially universal, residual DBSS combined with patient registries are a valuable resource for epidemiological research (1). The usefulness of DBSS is limited by the small amount of blood available, however, and methods that optimize the use of the sample are required, such as various whole genome amplification (WGA) methods used in genetic epidemiological studies (2, 3). We previously described a high-capacity multiplex immunoas-

say, based on Luminex xMAP technology that uses two 3-mm punches from each DBSS, for simultaneous determination of 25 inflammatory markers and neurotrophins (4). We now report that after extraction of proteins for the immunoassays, there is sufficient genomic DNA (gDNA) on the used DBSS disks to perform several WGA reactions, each producing enough whole-genome-amplified DNA (wgaDNA) for numerous reliable genotypings.

Blood from 20 anonymous volunteers was spotted on filter paper and air dried, and six 3-mm disks were punched out from each DBSS. Half the disks were subjected to protein extraction (4). The 2 groups of disks from each individual were then further split into 2 subgroups with, respectively, 1 and 2 disks in each, and gDNA was extracted in a volume of 200 μL using the Extract-N-Amp™ reagent set (Sigma-Aldrich). The concentration of gDNA (0.2 mg/L, measured by the Quant-IT™ PicoGreen® dsDNA Reagent) was not correlated to the use of 1 or 2 disks or to prior protein extraction. We then amplified extracted gDNA with 2 different WGA reagent sets: the GenomePlex Whole Genome Amplification Kit (Sigma-Aldrich), which is based on the Omniplex method, and the AmpliQ Genomic Amplifier Kit (Ampliqon), which uses the multiple-displacement amplification approach. Allowed input volumes are 10 μL for the GenomePlex (~2.0 ng gDNA) and 8 μL for the AmpliQ (~1.6 ng gDNA). Both reagent sets use less input gDNA than the recommended 10 ng, a feature that may be critical for the genotyping performance of the resulting wgaDNA (5), and thus direct comparisons cannot be made. The yield of wgaDNA was 2.6–3.8 μg, independent of whether protein extraction was performed and whether 1 or 2 disks were used.

The quality of wgaDNA was assessed by TaqMan® single-nucleotide polymorphism (SNP) genotyping of 27 SNPs in 27 genes distributed on 13 chromosomes. Reference gDNA was extracted from 200 μL of whole blood from the same 20 individuals. Genotype calls were

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References


done independently by 3 persons. The call and error rates are shown in Table 1. Despite the suboptimal amount of input gDNA, the best call and error rates were nearly optimal. The most common errors, results indicating that heterozygous individuals were homozygous, were attributable to allele dropouts most probably due to an insufficient amount or low quality of template gDNA. Details of sample flow and call and error rates for each individual gene can be seen in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue5.

When extraction of proteins from disks was performed before extraction of DNA, the resulting wgaDNA generally showed higher TaqMan genotype call rates and lower error rates than wgaDNA from disks that had not undergone pre-extraction of proteins (Table 1). This result suggests that the pre-extraction procedure removes compounds from the filter paper disks that may interfere with the WGA reaction. This explanation is in accordance with the observation that the use of 2 disks that had not undergone protein pre-extraction gave inferior results than did the use of only 1 disk, whereas the opposite was the case for the pre-extracted disks, for which the use of 2 disks instead of 1 yielded superior results (Table 1). The DBSS used in this study were stored for only a limited time, and we do not know the effect on the quality of wgaDNA of prolonged storage at room temperature or at −20 °C.

Our results demonstrate that DBSS disks previously used for multiplex protein measurements are reliable sources of gDNA that is suitable for WGA and SNP genotyping. The study also shows that both the Omiplex-based method producing short wgaDNA of 400–500 bp and the multiple-displacement amplification-based method producing long wgaDNA of 10–20 kbp are well suited for the amplification. In our setup, the amount of template gDNA from each extraction was sufficient for ~20 amplifications, and the yield of wgaDNA from 1 amplification was enough for ~300 TaqMan genotyping.

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References

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Pyrosequencing Technology for Automated Detection of the BMP15 A180T Variant in Spanish Postmenopausal Women

To the Editor:

Germline mutations in different genes are associated with premature ovarian failure (POF, OMIM 311360), defined as premature menopause with amenorrhea occurring before the age of 40 years along with increased gonadotropin concentrations [follicle-stimulating hormone (FSH) >40 IU/L]. A new candidate gene, bone morphogenetic protein 15 (BMP15) has been investigated in POF. In a family affected by hypergonadotropic ovarian failure, a mutation in the pro-region of the BMP15 gene (Y235C) was found in 2 affected sisters (1), and 3 linked markers within the BMP15 gene (−673C>T, −9C>G and IVS1 + 905A>G) are associated with high follicle production in women undergoing recombinant FSH stimulation (2).

Several heterozygous variations affecting the pro-region and mature peptide of the BMP15 gene have been identified in women with POF (3–5), but A180T was the only variant found in all reported studies, occurring with relatively high frequency in POF women (8 of 502, 1.6%) but not at all in control groups.

To clarify the role of the A180T allele in early menopause and ovarian failure, we used a pyrosequencing protocol (Biotype) to evaluate the A180T variant. This technology allows an easy 96-well typing format. The selected primers for pyrose-