Chapter

Detection of Nutrient-Related SNP to Reveal Individual Malnutrition Risk

Junsheng Huo and Chunhong Zhang

Abstract

Malnutrition is a result of complicated reasons from diet and food behavior and also related to genetic background which has been revealed by studies in recent decades. Traditionally, nutrition status are measured and expressed with indexes of anthropometric, diet survey, clinical symptom, biochemistry, behavior, etc. These measurement has been used in national nutrition monitoring, clinic nutrition therapy, mother and children nutrition care, nutrition intervention projects, and scientific studies. However, genetic and epigenetic information on nutrition explain malnutrition in a genetic view that would supply additional new theory and methodology for the growing requirement in terms of personalized and precise nutrition. In this chapter, an introduction on the detection of nutrient-related SNP to reveal individual malnutrition risk is discussed.

Keywords: SNP, nutrients, malnutrition

1. Introduction

Malnutrition is a state of disordered nutrition, in which a combination of varying degrees of over- or undernutrition and inflammatory activity has led to a change in body composition, diminished function, and outcome [1]. Malnutrition, including undernutrition, micronutrient deficiencies, and overweight and obesity, not only affects the people's health and well-being by impacting negatively on human physical and cognitive development, compromising the immune system, increasing susceptibility to communicable and noncommunicable diseases, restricting the attainment of human potential, and reducing productivity but also poses a high burden in the form of negative social and economic consequences to individuals, families, communities, and states [2].

Currently, more than 810 million people worldwide are hungry, mainly in poor, natural disaster-destroyed and war-torn countries. About 2 billion people are suffering from micronutrient deficiencies, which is called hidden hunger, and about 2 billion adults are affected by overweight and obesity, with one in 12 adults suffering from diabetes and one in two with cardiovascular diseases [3]. Developmental Origins of Health and Disease (DoHaD theory) considers that adult disease stems from malnutrition in the fetus and early childhood. More evidence accumulated that malnutrition would result in adverse consequences to the later life cycle and should be addressed in the whole life cycle [4].

Malnutrition is a result of complicated reasons from diet and food behavior and also related to genetic background which has been revealed by studies in recent decades. Traditionally, nutrition status are measured and expressed with indexes of anthropometric, diet survey, clinical symptom, biochemistry, behavior, etc. [5]. These measurement has been used in national nutrition monitoring, clinic nutrition therapy, mother and children nutrition care, nutrition intervention projects, and scientific studies. However, genetic and epigenetic information on nutrition explain malnutrition in a genetic view that would supply additional new theory and methodology for the growing requirement in terms of personalized and precise nutrition. In this chapter, an introduction on detection of nutrient-related SNP to reveal individual malnutrition risk is discussed.

2. Malnutrition related to inheritance

Following the restrictive enzyme cut technology on fragment length polymorphisms and short series repeat sequences, single nucleotide polymorphisms (SNP) became the third-generation polymorphism marker with the characteristics of high genetic marker density, high stability, and high feasibility of automation detection, which showed a strong application prospect in human genomics research, such as genetic diagnosis, genetic risk assessment, chain imbalance map, and genetic association analysis. Severe malnutrition such as iron deficiency anemia, xerophthalmia and nyctalopia, pellagra, scurvy, rickets, beriberi, and other nutrient deficiency diseases was caused by the combined impact of environment and genetic factors. And the Human Genome Project study showed that 99.9% of DNA sequences were consistent among different individuals, with only small genetic differences in the sequence. 0.1% of DNA sequence differences may vary the level of risk of malnutrition and diseases such as non-chronic diseases. Single nucleotide polymorphism could be measureable markers to reveal the genetic differences.

2.1 Iron deficiency-related genes

The discovery of polymorphisms on DNA sequences associated with common diseases was an important way to understand the risk of nutritional deficiency from genetic perspective. Iron deficiency was one of the most important nutritional problems in the world, especially in developing countries. Iron deficiency not only leads to anemia but also causes the body's immune function, work performance, and damage of adolescent's psychological behavior and mental development. With the deepening of research on nutritional genomics, genetic polymorphisms associated with iron nutrition status have been found. A study reported by Mclaren et al. [6] showed that rs2111833 and rs1121312 in TMPRSS6 gene with iron biochemical indicators showed that rs2111833 is associated with serum iron and log-to-ferritin saturation in the Caucasian population and shows total iron binding force, unsaturated iron binding force, and serum iron in the Asian population. Rs1421312 sites were associated with serum iron and log-to-ferritin saturation in the Caucasian population and serum iron and log-ferritin saturation in the Afro-American population. The study found that rs2111833 and rs1421312 had an impact on iron nutrition in different races.

3. Folic acid deficiency genes

Folic acid was a cofactor that interacted with a variety of enzymes in many intercellular reactions, with methionine enzymes acting as coenzymes when isocysteine

was converted to cystic thiopental. The extent to which the body absorbed folic acid and vitamin B_6 and B_{12} is influenced by environmental and genetic factors. In 1964, Smithells et al. [7] showed that women with reproductive neural tube malformations (neural tube defect, NTD) had micronutrient deficiencies, especially folic acid. NTDs were congenital malformations of the brain and spinal cord that occurred within pregnancy from 21 to 28 days, including spina bifida, anencephaly, and brain bulging, which could lead to infant death and child disability. NTDs had the epidemiological characteristics of environmental and genetic factors. In 1995, a variant of MTHFR enzyme was identified which causes a substitution of C to T at nucleotide 677 [8]. The MTHFR C677T homozygous variant (TT genotype) is thermolabile, and its activity is reduced by 70% compared to the wild type (CC genotype). This reduced enzyme activity causes an accumulation of plasma homocysteine and higher rates of thymidylate synthesis. It is well established that B vitamin status is affected by genotype, particularly the C677T polymorphism in MTHFR, with the T allele being associated with higher circulating concentrations of homocysteine and lower circulating concentrations of plasma and erythrocyte folate. In 2018, Zhang et al. [9, 10] explored the association between maternal methylenetetrahydrofolate reductase (*MTHFR*) C677T, methionine synthase reductase (*MTRR*) A66G, and methionine synthase (*MTR*) A2756G which effects on absorption and utilization of folate, B_6 and B_{12}, and neural tube defects in offspring through meta-analysis, which showed that these SNPs were significantly associated with NTDs in offspring. A cross-sectional study of dietary and genetic predictors of blood folate levels in a large racial healthy young adults group by Daniel et al. in 2017 [11] showed that the interactive effect of the genotype with naturally occurring food folate intake on RBC folate levels occurred in the anticipated stronger individuals that is homozygous for the T allele. This pattern suggests that polyglutamated folic acid (naturally occurring food folate) is less well absorbed among C allele carriers. This interpretation is consistent with the results from previous research, which found that those with hypofunctional *FOLH1* 484 variants had lower RBC folate levels despite equivalent dietary folate intake. Understanding why circulating folate levels vary from person to person is critical to ensuring adequate bioavailability, especially among women of childbearing age.

3.1 Genes of other nutrients deficiency

In recent years, the levels of folic acid; vitamin B_2, B_3, B_6, and B_{12}; and homocysteine (HCY) in pregnant women, as well as enzymes in folic acid and HCY pathways such as methylenetetrahydrofolate reductase (MTHFR), methionine synthase reductase (MTRR), and gene polymorphism sites associated with methionine synostic ingendraase (MTR) have been explored as potential causes of NTDs. Wilcken et al. [12] showed that the frequency of the homozygous C677T genotype (TT) was highest among individuals of Hispanic ethnicity, followed by whites, with the lowest frequency found in blacks. There were geographical and racial differences in gene polymorphisms, so future studies should conduct large samples and cross-regional surveys and established a database of gene polymorphisms in different regions and populations to provide a scientific basis for precise nutrition guidance and intervention.

3.2 Measurement of malnutrition-related SNPs

Nutrigenomics studies have sufficiently accumulated data in the last two decades to reveal phenotypes of SNPs between health and micronutrient deficiency population [13]. Zhang et al. [9, 10] explored that the genes of MTHFR C677T,

MTRR A66G, and MTR A2756G were genetic factors for low absorption and bioavailability of folate, B_6, B_{12}, etc. Those nutrients are closely related with the prevalence of neural tube defects (NTDs) in newborn infants. Daniel et al. [11] reported that mutant genotype of a C allele SNP of individuals predicted lower RBC folate concentration than that of T allele SNP of individuals with the same diet folate intake level. Studies have reported numbers of micronutrient deficiency-related SNPs (MD-SNPs) of vitamins A, D, E, B_6, and B_{12}, folate, calcium, iron, zinc, selenium, etc. [14–22]. It is assumed that MD-SNPs based on high -quality observations in large population could be used as biomarkers for assessing genetic potential risk of micronutrient deficiency. The sequencing of the human genome has catalyzed efforts to search for disease genes by the strategy of associating sequence variants with measurable phenotypes. In particular, the Human Genome Project and follow-on efforts to characterize genetic variation have resulted in the discovery of millions of SNPs, which have emerged as genetic markers of choice because of their high-density and relatively even distribution in the human genomes**.** When one nutrient deficiency risk-related gene has been mapped to a chromosomal region, a high-density SNP mapping or candidate gene association studies are logical steps to follow.

3.3 Gene sequencing

The first-generation sequencing technology, also known as Sanger sequencing method, is based on the sequencing of DNA polymerase synthesis reaction. The basic principle is that the test DNA template, desired DNA synthase, deoxynucleoside triphosphates (dNTPs), reaction buffer, primers, and other components of DNA synthesis reaction and a small amount of four kinds of radioisotope dideoxynucleoside triphosphates (ddATP, ddTTP, ddCTP, and ddGTP) were added to the reaction system. Because the ddNTP dideoxyribose connected on the 3-carbon atom is not a hydroxyl group (-OH) but the hydrogen (H) after deoxidation, the ddNTP is added to the DNA strand being synthesized, the system subsequent to dNTP no longer be bound to this DNA strand, and the synthesis of this DNA strand was randomly terminated at the base of the ddNTP. Thus, after several cycles, a group is formed from short to long DNA fragments; these fragments can be directly length difference of one nucleotide, and the 3‘end nucleotide is radiolabelled with A, T, C, or G. The product was divided into A, T, C, and G, the four electrophoresis lanes; the base can be read in the order to be synthesized, thereby obtaining the DNA sequence to be tested. Thereafter, on the basis of “the Sanger sequencing method,” the automatic detection and fluorescence techniques, isotopically labeled with a fluorescent label in place of the four fluorophores, four bases were replaced and automatically detected by imaging techniques, no longer subjected to electrophoresis separately read sequences, greatly improving the speed and accuracy of DNA sequencing. Generation sequencing technology to ensure smooth implementation of Human Genome Project can be obtained secret of human health and disease at the molecular level. However, first-generation sequencing technology has considerable limitations, namely, low throughput, high cost, and long time. Further, since test DNA Sanger sequencing is applied to the support, and cloned in *E. coli* and other bacteria, therefore, it could not be cloned fragments of harmful bacteria, and, in some regions of the genome, such as the centromere and terminal area around the particles is difficult to be cloned, leading to deletion of part of gene sequence. Additionally, the method of analysis is limited ability alleles, SNP detection is very difficult, which facilitates the birth of a new generation of genome sequencing technology.

3.4 Methods detecting SNP

Some technologies such as mass spectrometry, electrophoresis, and microarray hybridization are much more dependent on PCR multiplexing than others to reach their throughput potential [23–25]. However, the efficiency of these technologies was constricted by cross impact in the PCR of primers and DNA samples in one reaction tube. It is evidenced that less than 20 primer pairs could be amplified together that could not support large numbers of SNP measurement. And when multiple SNPs are amplified together in a reaction chamber, only 50–70% SNPs can be amplified successfully, and the amount of products varies greatly from 10 to 1000 folds, what leads to the cooling rate for samples which decreases dramatically—some SNPs that are scored for sample A may not be scored for sample B or C [26–28]. Single-base extension based on multiplexing PCR like mass spectrometry assay is expensive and requires well-trained personnel for performing the various steps of the analysis with a lengthy protocol. For most homogenous detection formats like fluorescence resonance energy transfer and fluorescent polarization, because their testing equipment have very limited capacity of multiplex recently, some technologies such as TaqMan 5′-nuclease assay, DNA hybridization, could not rely on multiplexing PCR to increase their throughput. Genotyping technologies have become a significant bottleneck for these applications despite rapid progress in the field. Exploring fast, accurate, high-throughput SNP genotyping, new technology is particularly urgent. Microfluidics chip is composed of microdroplets, microchannels, and microchambers [29–34]. Each microchamber could be used to amplify only one primer pair. A number of microchambers can be designed to meet the requirement. The physical isolation of different primer pairs is a simple and effective strategy to avoid the drawbacks of conventional multiplex PCR. In order to overcome the SNP genotyping error @ caused by different amplification efficiency, chambers and channels with special structures have also been designed for primer storage and allocation of reaction mixtures. The characteristics of smaller reaction volumes, high-throughput capacity, ease of integration, and portability compared to traditional PCR endow microfluidics (microdroplets, microchannels, and microchambers) with the potential to be a powerful technology to meet SNP genotyping demands. For example, 116-plex PCR designed by Li et al. can be accomplished using a hydrophobically patterned microarray [35]. However, it requires precise operations, and the amplification is performed in an open environment, which risks contamination. Microdroplets (e.g., digital PCR) are also a potential technology for multiplex PCR, but barcoding technology is essential but challenging for multiplex digital PCR. The OpenArray® platform from Applied Biosystems, which was the commercial products for multiplex PCR, is rather expensive and sophisticated, and costly instruments are also required. In brief, these methods for multiplex PCR are effective, but complications in chip processing and/or their high associated costs hinder their wide use.

3.5 Measurement malnutrition-related SNPs and their genotypes

SNP genotype measurement has been widely studied in the risk screen and diagnosis for genetic diseases and chronic diseases, but few studies for nutrient deficiency risk determination. It is agreed that the body micronutrient diagnosis or evaluation is the bottleneck technology barrier since there are numbers of indexes for varieties of micronutrients; in addition, there seems even difficulty to know the genetic information related with micronutrient deficiency. Nutritional genotype studies have facilitated to use MD-SNPs (malnutrition-related SNPs) as risk biomarkers, i.e., vitamins A, D, E, and B_{12}, folate, calcium, iron, zinc, and selenium.

3.5.1 Microfluidic chip designed for malnutrition-related SNPs

Microfluidic chip as high-throughput technology could amplify large numbers of target DNA fragments at the same time in a chip, and the physical isolation of different primer pairs is a simple and effective strategy to avoid the drawbacks of conventional multiplex PCR. Xu et al. took this advantage to reduce the mutual interference and competition among different primers in one tube for multiple PCR [36]. They adopted a modified method with a blocking step, which had showed less contamination than that without blocking method. A study reported by Zhang et al. showed that MD-SNPs were extracted from published studies of GWAS, reviews, and meta-analysis, which epidemically related with micronutrient deficiency, and a method was established by modified microfluidic chip for MD-SNPs measurement by Xu et al. The study would explore possibility to describe MD potential risk from genetic point of view for an individual.

3.5.2 Primer design of nutrition-related SNPs

Primer mix contained three primers, common reverse primer, tailed allele primer 1 and tailed allele primer 2, in a ratio of 5:2:2. Primer mix (0.14 μl, 2 μM for each forward and reverse primers) was preloaded in a reaction chamber. Master mix containing FRET cassette plus enzymes with high-fidelity activity in an optimized buffer solution was stored at −20°C in the refrigerator, kept cool with ice when taken out from refrigerator, and vortex shocked before use (**Figure 1**).

3.5.3 MD-SNP measurement process

The material of the chip was polymethylmethacrylate (PMMA) and was fabricated by machining to final dimensions of 7.5 cm (length) × 2.5 cm (width) × 2 mm (thickness). There were 28 microchambers in a column and 4 parallel columns in a chip that supported for simultaneously testing of 112 SNPs in 3 genotypes of wild type, hybrid type, and mutant type. Each column consisted of a circular inlet and outlet, a "sine-shaped" sample infusing channel, 28 linking channels, and 28 circular reaction chambers. A modified method has been established to prepare the microfluidic chip. Prior to use, the chip was washed with ethanol and ultrapure water and dried with nitrogen gas. Then, the primer pairs were pipetted into different reaction chambers and allowed to dry at room temperature for 30 min. A piece of single-sided, PCR-compatible adhesive tape was used to seal the top side of the chip at 175°C for 1 min. After sealing, the primer-loaded chip was stored at 4°C before use. An aqueous PCR mixture containing PCR master mix and DNA template was loaded into infusing channels by pipetting from the inlets. Outlets and inlets on the bottom side were sealed with adhesive tape to achieve a fully hermetic system. Then, the chip was centrifuged at 4000 rpm for 1 min so that the PCR mixture was uniformly transferred into reaction chambers and thoroughly mixed with the preloaded primers mix, and the final reaction volume was 0.8 μl. Each linking channel was blocked at 150°C for 1 min. Then, the chip was placed on a MasterCycler Nexus flat and pressed with a PMMA block to ensure tight contact and avoid distortion of the chip under high temperature (**Figure 2**).

The temperature program of PCR in the chip was set as follows (**Figure 2**): hot-start activation at 94°C for 15 min, followed by 10 touchdown cycles (94°C for 20 s; touchdown 61–55°C, dropping 0.6°C per cycle), and then followed by 26 cycles of amplification (94°C 20 s; 55°C 60 s). After thermal cycling for 100 min, the amplified products were detected by LuxScan-10 K/A scanner at 40°C or below for

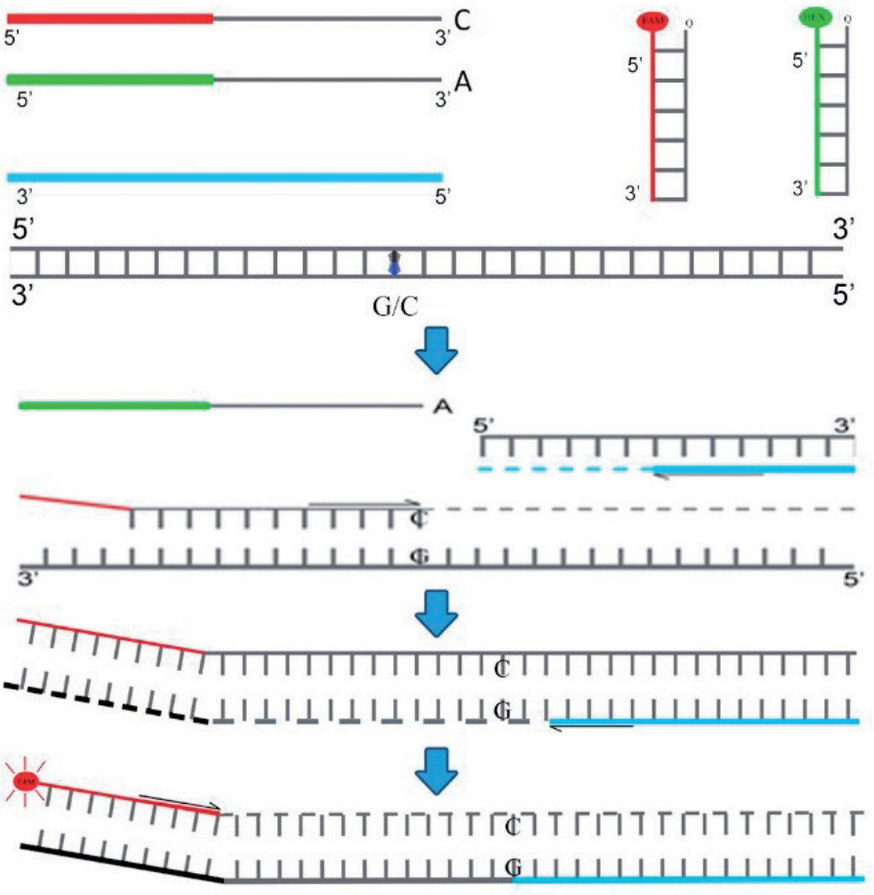

Figure 1.
Steps and principle of allele-specific extension on primer arrays. (A), primer pairs mix containing two different, allele-specific, competing forward primers with unique tail sequences and one reverse primer. Master mix containing FRET cassette plus enzymes with high-fidelity activity in an optimized buffer solution. Test DNA with the SNP of interest. (B), in the first round of PCR, one of the allele-specific primers matches the target SNP and, with the common reverse primer, amplifies the target region; (C), in the second round of PCR, reverse primer binds, elongates, and makes a complement copy of allele-1 tail; (D), in the third round of PCR, FAM-labeled oligo binds to new complementary tail sequence and is no longer quenched; in further rounds of PCR, the levels of allele-specific tail increase. The fluorescent substance-labeled part of the FRET cassette is complementary to new tail sequences and binds, releasing the fluorescent substance from the quencher to generate a fluorescent signal.

15 min. The fluorescence intensity values (FIVs) were used to identify three distinct genotypes of wild type, hybrid type, and mutant type.

3.5.4 Cross-contamination test

Odd-numbered chambers in a column of a chip were preloaded primer mix, while even-numbered chambers were not. In addition, gel electrophoresis was observed with solutions from the corresponding reaction chambers. For specificity of primer mix and accuracy, each primer pair preloaded chamber loaded different template DNAs in a concentration of 10 ng/μl with master mix pipetted into infusing channels. The results were compared with the expected results obtained by next-generation sequencing (NGS). For the selection of appropriate DNA reaction concentration, 52 difficult DNA templates were diluted to 1 ng/μl, 5 ng/μl, 10 ng/μl, and 15 ng/μl to test appropriate DNA reaction concentration, respectively. The repeatability of multiplexed SNPs is observed with four repeats of 52 MD-SNPs in one DNA template. All these experiments were repeated six times. The established method was used to measure DNA templates from six

different samples to evaluate the possible MD risk of vitamin A, D, E, and B_{12}, folate, calcium, iron, zinc, and selenium (**Figure 3**).

3.5.5 Multiplex PCR in MD-SNP measurement

Multiplex PCR is a promising method for multiple nucleic acid analysis and detection. Several primers involved in a single tube behaves as multiplex PCR, in which one allele sequence is often preferentially amplified, resulting in the scarcity of other allele sequences, so it is very tedious to establish an optimized multiplex PCR protocol. Most chip-based multiplexed genotyping platforms are suitable for large-scale studies requiring genotypic data with thousands of SNPs. Although

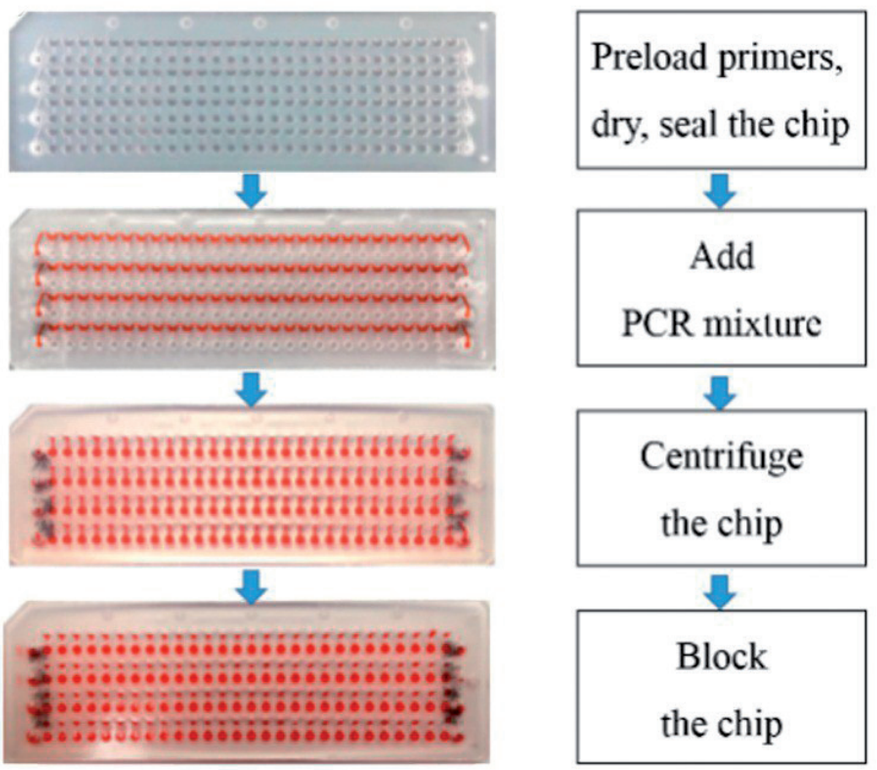

Figure 2.
Workflow protocol of the chip.

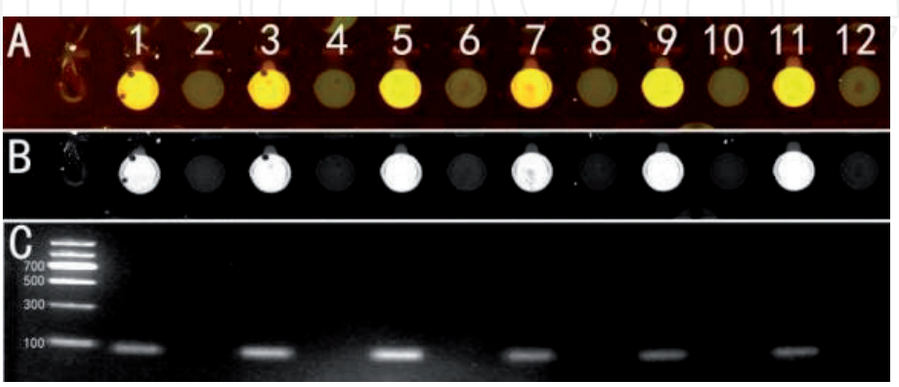

Figure 3.
The cross-contamination testing of adjacent reaction chambers. Odd number and even number represented reaction chambers with and without preloaded primers, respectively. (A) is the fluorescence pseudo-color image; (B) is the corresponding gray scale image; (C) is the electrophoretogram of the amplicons in each reaction chamber which corresponded to the product in the chamber of (A) or (B) above. The lane marked with M represents the DNA marker. The molecular weights of the bands from the top to bottom were 1200, 900, 700, 500, 300, and 100 bps.

multiplexing offers greater throughput with less reagent consumption, it restricts the use which require low to medium marker density, for example, Illumina company requires a minimum number of plates ordered in order to develop specific assays, and this requirement was much higher than individual needs. So Xu et al. developed an innovative microfluidic chip to be the most convenient and cost-effective option for genotyping various individuals, which physically isolates the primer pairs in a reaction chamber. Fifty-two SNPs demonstrated the effectiveness of our strategy by multiplex PCRs and further illustrated its clinical applicability with blood and saliva samples. As a qualitative method, primer pairs of MD-SNPs designed in this study could be successfully amplified in the given conditions and replicated target DNA fragments with additional florescence carriers of FAM and HEX. Three genotypes of mutant type, hybrid type, and wild type could be identified specifically and accurately by the measurement. The sample chambers showed averagely at least two times higher FIV than that of NTCs. 5 ng/μl or higher suggested the suitable DNA concentration for the selected 52 MD-SNPs, although the optimal concentration for each primer pair may be different. The method showed high repeatability in both inner chips and among chips. The results of 52 MD-SNPs determined by MD-chips and NGS were completely the same, suggesting a high accuracy of the method.

There are several advantages of the microfluidic chip multiplex PCR: (1) the generality of the primer design principle which was adopted by this microfluidic chip assays. The principle is developed for common use in all genotyping assays to stringently target the two alleles with standard PCR conditions and similar amplification efficiencies and significantly decreases the cost in PCR reagents and labors. (2) It is more specific. The microfluidic chip genotyping results were completely coincident with next-generation sequencing results. (3) It is easier. All the primer pairs are physically isolated; deleting or adding one or a few primer pairs from a multiplex PCR primer panel will not alter the performance of the other primer pairs with standard PCR conditions; almost no additional optimization is required. So, the universal protocol is viable for developing diverse multiplex PCR applications. (4) Its throughput is flexible. The selection of a technique has to weigh factors of instrument, throughput, technical support, and cost. Because of its unprecedented specificity, simplicity, and flexibility of throughout, the chip could serve as a powerful tool in clinical individual nutriment deficiency risk diagnostics for multiplexed detection of nutriments. The results of the analysis performed using the chip may provide early and crucial information for physicians to prevent nutriment deficiency risk and conduct appropriate nutritional intervention.

4. Malnutrition risk evaluated by personal SNPs

4.1 Expression of malnutrition risk with MD-SNPs

The MD-SNP chip method was used to measure MD risk of six students which showed distinguished differences of genetic potentials. The MD risk of six students was shown in a colored image in a pattern of SNP genotypes in three colors. The wild type was in red, hybrid type in orange, and mutant type in green. The risk of individuals in a micronutrient deficiency could be identified by the differences of red color areas. The genotype for each SNP of micronutrients in a person could be presented with the image (**Figure 4**).

4.2 Guiding on nutrient intake with MD-SNP

The combination of this method with present laboratory measurement might comprehensively explain individual MD risk in both genetic and diet environmental

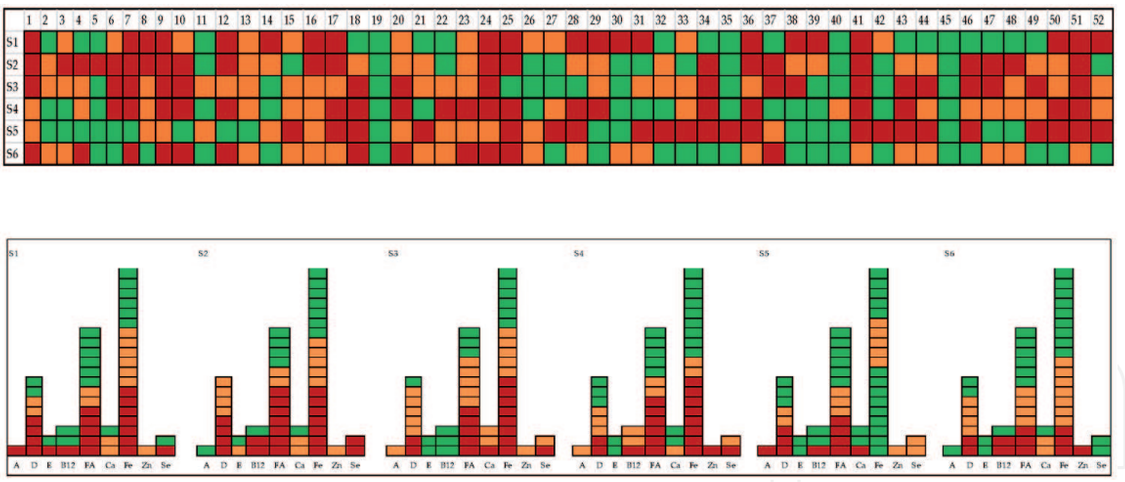

Figure 4.
The color grade of nine MD-SNPs in six measured individual samples. S, sample.

conditions, thus facilitating with precise nutrition intervention. For example, MTHFR-C677T polymorphism has been extensively studied, and the association between the TT genotype and low folate status is well documented. Individuals with the TT genotype seem to be particularly susceptible to insufficient status of several B vitamins, and they may need to consume more folate to maintain serum folate levels similar to those found in individuals with the CC/CT genotypes. A study by Crider et al. reported that daily 0.8 mg folic acid may be necessary to lower homocysteine concentration for Chinese hypertensive subjects with CT or TT genotype, which have important clinical and public health implications [37]. The Centers for Disease Control and Prevention in the United States showed in 1992 that women who have previously suffered a NTD-affected pregnancy are advised to take 4 mg of folic acid daily before conception and during the first months of pregnancy [38]. Then we could calculate the required amount of an individual micronutrients according to SNPs.

In the real world, the body's nutritional status is regulated by multiple genes and nutrients. For example, homocysteine (Hcy) is a precursor of methionine and cysteine. Methionine converts into S-adenosyl methionine, which acts as a universal methyl donor. These multistep reactions involve various enzymes and cofactors in the form of essential micronutrients, which include vitamin B complex family (B_2, B_6, B_9, and B_{12}). Therefore, in measuring tHcy, folic acid and vitamin B_{12}, vis-a`-vis the genotypes of the Hcy-pathway genes, Zhang et al. evaluated contribution of the individual variables (SNPs of Hcy-pathway genes) in the development of the phenotype (Hcy level) and get an estimate of the relative contribution of the environment (vitamins) in modulating the effect of genotypes in this region. Hyperhomocysteinemia is a result of either reduced enzymatic activity in the enzymes that participate in homocysteine metabolism and/or a reduction in the concentrations of plasma B vitamins, particularly, folate. Dietary intake of folate or folic acid supplementation can lower the concentration of p-tHcy. The establishment of gender and age as covariates is associated with SNP HCY polygenic risk score model (PRS), PRS = −0.024802rs2274976 + 0.025011rs1801131 + 0.205567rs1801133–0.025646rs1805087–0.025047rs2118981 + 0.340703rs492602–0.448651rs602662 + 0.067954sex + 0.060073 age+1.553543; sex, female 0, male 1; age, Year (R^2 = 0.4084, p < 2.2e-16).

The incidence of early detection, prevention, and intervention was the fundamental goal of promoting human health; predicting the probability of an

individual assessment of the risk of susceptibility to disease was the core clinical decision-making, especially for the detection and prevention of common diseases. The current clinical data for common adult disease risk often relied on basic human indicators, such as age, gender and ethnicity, lifestyle, and basic health indicators, such as body mass index, smoking status, alcohol use, and physical activity habits; suffering disease relevant to the biomarkers like blood pressure level and biochemical indexes; analysis of environmental exposure, such as air pollution, heavy metals, and other environmental toxins; and family history. Many recent studies have begun to demonstrate the utility of gene association analysis and access to individual genetic susceptibility to a disease useful for the guidance of information from the probability of large population data. In theory, gene mapping could be considered a useful part of a healthy management.

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