ABO Blood Groups Genotyping in Palestine

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Abstract— **ABO blood group genotyping is an area of current research interest, as ever more rapid and direct methods are sought for forensic and medical applications (Lee, et al 2011), but molecular blood group genotyping is absent in Palestine. We worked to fill this gap by surveying national blood group distribution amongst blood donors and developing our own molecular assay for medical uses in Palestine.**

Keywords: ABO genotyping. ABO blood system. PCR.

I. INTRODUCTION

The international society of Blood Transfusion (ISBT) approved 29 blood group systems (Hosoi, 2008) that are based on more than 250 antigens (Reid M M. K., 1998). The key features of these systems and their antigens were described by Reid and Lomas-Francis in 1997 (Reid M L.-F. C., 1997). ABO is one of the major systems; (Nishimukai, 1996) it consists of four antigens (A, B, AB, and O) (Hosoi, 2008), while another study described the fourth antigen as A1 instead of the O (Mokhtar M. El-Zawahri, 2008). The ABO system was discovered by the Austrian scientist Karl Landsteiner in 1900 (Hosoi, 2008).

The major A, B and O antigens are coded by the A, B and O major alleles, respectively, at the ABO locus on the human chromosome 9 at 9q34.1–q34.2 (Mokhtar M. El-Zawahri, 2008) and the molecular genetic basis of the major alleles at the ABO locus has also been established (Yamamoto F M. J., 1990). The ABO locus is over 18 kb and consists of seven exons that make a galactosyl transferase of 354 amino acids that is responsible for adding a terminal n acetyl-galactosamine to an oligosaccharide blood group precursor antigen called 'H antigen' (Reid M L.-F. C., 1997). The exons range in size from 26 to 688 bp and most of the coding sequence is included in exon 7 (Yamamoto F C. H., 1990).

Many mutations affecting the kinetic efficiency and specificity of the encoded transferases have been identified (Yamamoto F H. S., 1990) . The nucleotide sequences of the A and B genes differ in seven base substitutions, but only four of these result in an amino acid change. The only difference between the O and A transferase genes is a single cytosine deletion, which results in a frame shift, producing a premature stop codon, leading to a truncated, enzymatically inactive protein of 115 amino acids. A second O allele (O2) that lacks this deletion also codes for a transferase that lacks enzymatic activity. The A and B alleles differ in their specificity for the terminal sugar residue to be attached to the oligosaccharide chain. The A2 allele has been characterized by a single base deletion in the coding sequence which results in an additional domain at the C terminus (Yamamoto F C. H., 1990).

Molecular cloning of the ABO gene and comprehension of the molecular basis of its various alleles paved the way for the direct determination of the ABO genotypes without the need for family pedigree analysis. A variety of assays for polymorphism detection at the DNA level have essentially replaced the former serological assays even for routine ABO typing for blood transfusion (Mokhtar M. El-Zawahri, 2008). Various methods have been used in the light of the increasing allelic variations. Several PCR based techniques exist, such as RFLP, which provided the first basis for studying genetic polymorphism at the DNA level (Lee J, 1992) and takes advantage of altered restriction enzyme recognition sites caused by single base substitutions responsible for the A, B and O alleles and sub-alleles. Other assays include allele-specific PCR (AS-PCR) (Ugozzoli L, 1992) , and there are others as well.

II. MATERIALS AND METHODS

A. Molecular Work

DNA was extracted from different type of samples, specifically buccal epithelial cells and blood. The DNA was extracted using the EZ-DNA extraction kit. 1ml of whole blood was added to 2ml RBC Lysis Solution (Cat. No. 01-888-1). It mixed gently at room temperature for 5 to 10 minutes. Then it was centrifuged at 300g for 10 minutes and the supernatant discarded. 1ml EZ-DNA solution was added, and the sample was mixed by repeated pipetting followed by incubation for 5 minutes at room temperature. 1ml of absolute ethanol per 1ml of EZ-DNA solution was added and the samples mixed by inverting the tubes 10 times. The samples were left for 3 minutes at room temperature. After the DNA become visible, it was extracted by spooling with a tip followed by centrifugation at 5,000g for 5 minutes. The DNA pellet was washed twice with 1ml 95% ethanol. In the first wash a solution containing 50% EZ-DNA solution and 50% ethanol was used. Washing was helped by inverting the tubes 10 times, after which the DNA left to settle to the bottom, and then the ethanol was removed and the DNA left for 5min to air-dry. The DNA was resuspended by adding 8 mM NaOH (fresh preparation). After storing for 5 minutes and the DNA was helped into solution by pipetting. A small variation of this

protocol was used to extract the DNA from the buccal-epithelial cells. The start was different because a swab was used to collect the cells for DNA extraction, which were suspended in 200 ul of EZ-DNA directly. The rest of the protocol was the same as that previously mentioned.

The extracted DNA was subjected to PCR in a total volume of 25µl using four primer sets of forward and reverse primers; the first pair was 1.**AGCTCCATGTGACCGCACGC** and **2.AATGGGAGCCAGCCAAGGGGTA.** The second pair 1. **CCAAGGACGAGGGCGATTTCTACTACC** the reverse of the second was **.GGTGGTTCTTGGGCACCGCA.** The third pair was 1. **CAGCTGTCAGTGCTGGAGGTGG**, the reverse was 2.**TGTAGGCCTGGGACTGGGGC.** The primers were designed depending on a deletion mutation and substitution mutation in exon7 [2].

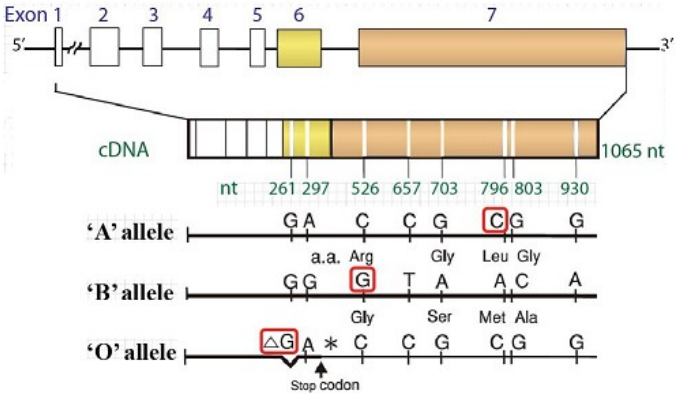

Figure 1: A: Exon arrangement of the gene. Exons 6 and 7 (shaded) carry most of the mutations. B: the major specific mutations and their resulting alleles.

The PCR reaction consisted of: 1µl of each primer, forward and reverse, and 2µl of the Template DNA. 12.5µl of 2X master mix was added and the total volume completed by addition of 8.5µl of ultra pure water. The PCR incubation was performed using a thermal cycler. The cycle conditions were as follow: initial denaturation for 5min at 95°C followed by 35 cyclesof (denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec). Final extension was carried out at 72°C for 7 min and the PCR products were stored in the thermal cycler at 4°C until they were collected.

B. Satistical Analysis

ABO blood groups distribution in Palestine was assesed. A raw dataset that represents the west bank district was collected from the Central Blood Bank in Ramallah, which kindly made available to us its records of 19,166 donations from 2006 through to 2011. In order to have a random sample, it was necessary to take care to overcome replicates that could skew the data towards "desirable" blood groups, such as O, which is considered a "universal donor" suitable for transplanting into any ABO group recipient. Replicate data from repeat donors were identified, on a balance of probability, as detailed personal identification was not available owing to ethical considerations. Our data set contained the name of the organization/company/university of the donor and his date of birth, which enabled screening out likely replicates by searching the data for identical birth-dates from the same organization. It was assumed, for the purpose of this study that donors did not move from one organization to another within the dataset. After the replicate data were removed a Palestinian blood group distribution was determined and compared with different countries that represent the West and the Far East.

III. RESULTS

A. Molecular Work Result:

We succeeded to distinguish between the blood group types at the molecular level, specially the B and the A groups. The figure below shows the specific band product of the AO/AA genotype and BO genotypes, which are inferred from the PCR product lengths of 276 bp for the primer pair that matches both the A and the O allele and the PCR product of 262 bp that is specific for the B allele only.

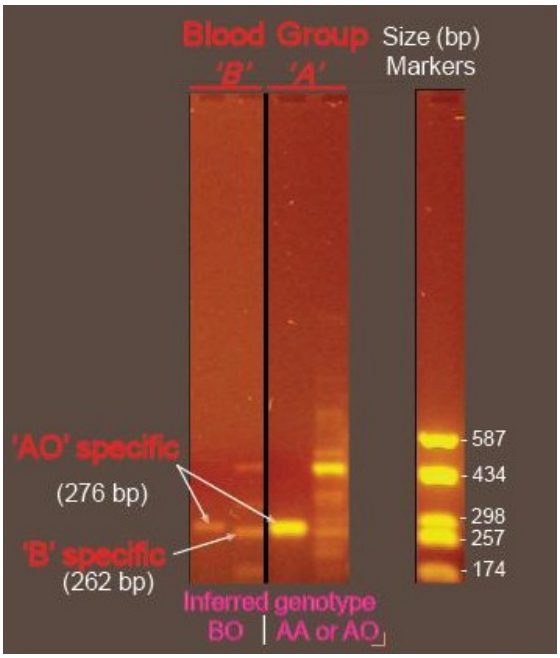

Figure 2: The PCR result shows a specific band for A and B genotypes

B. Satistical Analysis

The statistical result shows that the highest percentage in Palestine is the Blood group A and the least is the blood group AB. The figure below shows the percentage of the four main groups in Palestine and compares it with three major countries in the world. Japan represents the Far East and the United States of America and the United Kingdom represent the West. It is apparent that the blood group distribution in Palestine is somewhat intermediate between those of the West and Far East, but closer to that of Japan than to that of the USA and UK.

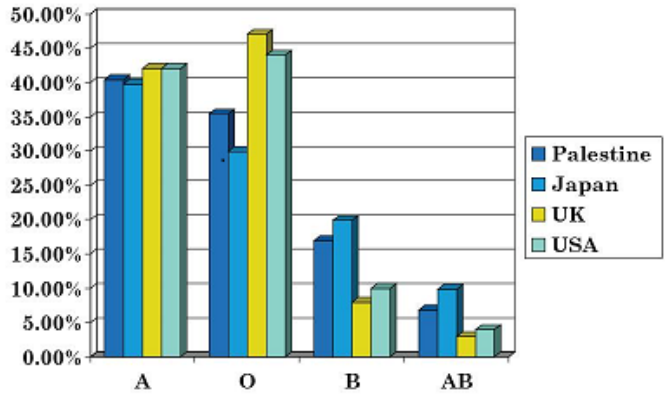

Figure 3: Comparison between Palestine and 3 different main countries in the world (Japan, United Kingdom and United States of America).

IV. DISCSSION

The work on the Blood groups genotyping succeeded to distinguish between the AO genotype and the BO genotype at the molecular level, but in order to distinguish between the four main groups and their subgroups, further work is needed.

As shown in the results, Palestine's blood groups distribution is intermediate between those of the West and Far East and follows the same overall trends where the A blood group predominates, followed by O then B and finally AB. Interestingly, the rarer B and AB groups are relatively highly represented by Palestinian and Japanese people suggesting a more closely intertwined evolutionary history than with peoples of the West. Of major concern is that availability of the "universal donor" blood group O is very much lacking in Palestine: there is a more than 10% difference between its occurrence in the UK, for example, and Palestine.

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