Blood Transfusion

Martha Roper

Blood Transfusion

Edited by **Martha Roper**

hayle
medical
New York

Published by Hayle Medical,
30 West, 37th Street, Suite 612,
New York, NY 10018, USA
www.haylemedical.com

Blood Transfusion
Edited by Martha Roper

International Standard Book Number: 978-1-63241-059-7 (Hardback)

Printed in China.

Preface

Blood transfusion is a process principally used to treat various medical conditions. This book deals with the applications of blood transfusion in diverse clinical surroundings. It discusses significant issues like the fundamental theories regarding ABO blood group system in blood transfusion, the use of transfusion in a variety of clinical surroundings, among others. It also deals with transfusion transmitted diseases, different techniques for allotransplantation and immunomodulatory effects of blood transfusion. The book covers various aspects of blood transfusion and will provide valuable information to its readers.

All of the data presented henceforth, was collaborated in the wake of recent advancements in the field. The aim of this book is to present the diversified developments from across the globe in a comprehensible manner. The opinions expressed in each chapter belong solely to the contributing authors. Their interpretations of the topics are the integral part of this book, which I have carefully compiled for a better understanding of the readers.

At the end, I would like to thank all those who dedicated their time and efforts for the successful completion of this book. I also wish to convey my gratitude towards my friends and family who supported me at every step.

Editor

Contents

	Preface	**IX**
Part 1	**ABO in the Context of Blood Transfusion**	**1**
Chapter 1	**ABO in the Context of Blood Transfusion and Beyond** Emili Cid, Sandra de la Fuente, Miyako Yamamoto and Fumiichiro Yamamoto	3
Part 2	**Blood Transfusion in Medicine and Surgery**	**23**
Chapter 2	**Blood Transfusion Practices in Major Orthopaedic Surgery** Saqeb B. Mirza, Sukhmeet S. Panesar and Douglas G. Dunlop	25
Chapter 3	**Transfusion Reduction in Orthopedic Surgery** Ruud H.G.P. van Erve and Alexander C. Wiekenkamp	51
Chapter 4	**Blood Transfusion Therapy in High Risk Surgical Patients** João Manoel Silva Junior and Alberto Mendonça P. Ferreira	73
Chapter 5	**Scope of Blood Transfusion in Obstetrics** Subhayu Bandyopadhyay	83
Chapter 6	**Uterine Atony: Management Strategies** Pei Shan Lim, Mohamad Nasir Shafiee, Nirmala Chandralega Kampan, Aqmar Suraya Sulaiman, Nur Azurah Abdul Ghani, NorAzlin Mohamed Ismail, Choon Yee Lee, Mohd Hashim Omar and Muhammad Abdul Jamil Mohammad Yassin	97

Chapter 7 **Effects of Blood Transfusion on Retinopathy of Prematurity** 129
Ebrahim Mikaniki, Mohammad Mikaniki and Amir Hosein Shirzadian

Part 3 Preventing Transfusion Transmitted Infections 141

Chapter 8 **Implicating the Need for Serological Testing of Borna Disease Virus and Dengue Virus During Blood Transfusion** 143
S. Gowri Sankar and A. Alwin Prem Anand

Chapter 9 **Transfusion-Transmitted Bacterial, Viral and Protozoal Infections** 163
Pankaj Abrol and Harbans Lal

Chapter 10 **Human T-Lymphotropic Viruses (HTLV)** 175
Marina Lobato Martins, Rafaela Gomes Andrade, Bernardo Hinkelmann Nédir and Edel Figueiredo Barbosa-Stancioli

Part 4 Alternative Strategies to Allogenic Blood Transfusion 189

Chapter 11 **Autotransfusion: Therapeutic Principles, Efficacy and Risks** 191
A.W.M.M. Koopman-van Gemert

Chapter 12 **Bloodless Medicine and Surgery** 209
Nathaniel I. Usoro

Chapter 13 **Impact of Acute Normovolemic Hemodilution in Organ and Cell Structure** 223
D.A. Otsuki, D.T. Fantoni and J.O.C. Auler Junior

Chapter 14 **Cryopreservation of Blood** 233
Miloš Bohoněk

Part 5 Immunomodulatory Effects of Blood Transfusion 243

Chapter 15 **Effect of Blood Transfusion on Subsequent Organ Transplantation** 245
Puneet K. Kochhar, Pranay Ghosh and Rupinder Singh Kochhar

Chapter 16 **Posthepatic Manipulative Blood Extraction with Blood Transfusion Alleviates Liver Transplant Ischemia/Reperfusion Injury and Its Induced Lung Injury** 255
Changku Jia

Permissions

List of Contributors

Part 1

ABO in the Context of Blood Transfusion

1

ABO in the Context of Blood Transfusion and Beyond

Emili Cid, Sandra de la Fuente,
Miyako Yamamoto and Fumiichiro Yamamoto
Institut de Medicina Predictiva i Personalitzada del Càncer (IMPPC), Badalona, Barcelona, Spain

1. Introduction

ABO histo-blood group system is widely acknowledged as one of the antigenic systems most relevant to blood transfusion, but also cells, tissues and organs transplantation. This chapter will illustrate a series of subjects related to blood transfusion but will also give an overview of ABO related topics such as its genetics, biochemistry and its association to human disease as well as a historical section. We decided not to include much detail about the related Lewis oligosaccharide antigens which have been reviewed extensively elsewhere (Soejima & Koda 2005) in order to focus on ABO and allow the inclusion of novel and exciting developments.

ABO group	A/B antigens on red blood cells	Anti-A/-B in serum	Genotype
O	None	Anti-A and Anti-B	O/O
A	A	Anti-B	A/A or A/O
B	B	Anti-A	B/B or B/O
AB	A and B	None	A/B

Table 1. Simple classification of ABO phenotypes and their corresponding genotypes.

As its simplest, the ABO system is dictated by a polymorphic gene (ABO) whose different alleles encode for a glycosyltransferase (A or B) that adds a monosaccharide (N-acetyl-D-galactosamine or D-galactose, respectively) to a specific glycan chain, except for the protein O which is not active. The 3 main alleles: A, B and O are inherited in a classical codominant Mendelian fashion (with O being recessive) and produce, when a pair of them are combined in a diploid cell, the very well known four phenotypic groups (see Table 1). Being one of the first known and easily detectable polymorphic traits in humans, it has been extensively studied as a historical background illustrates.

2. History

Various advancements in both blood storage and serology have contributed to the development of safe blood transfusion. One of the key events that brought transfusion

medicine forward was the discovery of the ABO blood group system. The first successful attempts of human to human blood transfusion already started in the 18th century, but it was an unsafe process in which some patients died. It was not until 1900 when the Austrian pathologist, Karl Landsteiner discovered the ABO blood group system, which opened the door for performing safe blood transfusions (Landsteiner, 1900).

Landsteiner separated the cell components and the sera of blood samples from different individuals, including his own blood, and mixed them in various combinations. He observed that in some combinations red blood cells (RBCs) agglutinated. According to these agglutination patterns, Landsteiner classified the individuals in three different groups. These blood groups were called A, B and C (later called the blood group O). One year later, Decastello and Sturli described one new group, the AB blood group (von Decastello & Sturli, 1902).

Landsteiner theorized that the RBCs possessed two different markers (antigens A and B) able to react with the corresponding sera antibodies (anti-A and anti-B), and as opposite to many other blood group systems such as the Rh system, the presence of the antibodies against A or B occurs naturally in individuals that do not express the antigens. The serum from an individual with A type RBCs present antibodies against the B antigen, so it is able to agglutinate B and AB type RBCs, but not his own type. The serum from B type individuals agglutinates A and AB type RBCs. Finally, the serum from O group can agglutinate A, B and AB RBCs because it contains both anti-A and anti-B antibodies while the serum of AB group do not have reactivity towards none of these antigens. This phenomenon was later known as Landsteiner's Law.

From that first discovery of the ABO system, new developments took place relatively fast during the following years. On 1908, Epstein and Ottenberg suggested that the blood groups could be an inherited character (Epstein & Ottenberg, 1908). That was confirmed in 1910 by von Dungern and Hirszfeld who showed that inheritance of the A and B antigens obeyed Mendel's laws (von Dungern & Hirszfeld, 1910). In fact, ABO was one of the first genetic markers to be used in paternity testing and forensic medicine.

To explain the mode of inheritance, Berstein proposed, in 1924, the one gene locus-three alleles model. He assumed that the A, B and O genes were alleles at the same ABO genetic locus and that the A and B alleles were co-dominant against the recessive O allele (Crow, 1993).

Already by 1926, it was shown that A and B antigens were not restricted to the surface of erythrocytes. They were also found in semen and saliva, and four years later, Putkonen and Lehrs discovered that the ability to secrete these antigens was genetically independent from the ABO gene and inheritable in a classical dominant Mendelian manner (Putkonen, 1930).

In 1950s, two research groups, one led by Kabat and another group led by Watkins and Morgan elucidated the chemical nature of ABH substances (H antigens were found abundantly in individuals with blood group type O) (Kabat, 1956; Morgan, 1960; Watkins, 1981). They determined that they were oligosaccharide antigens and also pointed out the biochemical difference between A (with a terminal N-acetylgalactosamine) and B (with galactose instead) substances. Moreover, they demonstrated that the ABO blood group system antigens were not the primary gene products (i. e. protein antigens), but were the result of enzymatic reactions producing carbohydrate chains. In the following years various works established the tissue distribution of these antigens and their changes during embryonic development (Ravn & Dabelsteen, 2000).

Between 1970 and 1980, the metabolic pathways leading to the biosynthesis of ABH antigens were established (Watkins, 1981) and in 1976, the ABO locus was localized and assigned to chromosome 9q34 (Ferguson-Smith et al., 1976).

In 1990, after the purification of the soluble form of human A transferase by Clausen and collaborators (Clausen et al., 1990), Yamamoto and his group were able to clone the cDNAs for A glycosyltransferase first (Yamamoto et al., 1990b) and afterwards those for the B glycosyltransferase and the O protein and elucidated the molecular basis for the synthesis of A and B antigens (Yamamoto et al., 1990a). Since the original description of the main alleles (A1, B, O) many others have been described by them and other groups and they have been annotated and included in public databases.

Together with the amino acid substitutions between A and B transferases, as well as the mutations causing a decrease or ablation of the enzymatic activity in A/B weak subgroup alleles and O alleles, the determination of their 3-D structure has facilitated a better understanding of the structure-functional relationship of these transferases (Patenaude et al., 2002).

3. Biochemistry and structure

A and B antigens share the same structure except for a terminal sugar bound by an α1-3 glycosidic linkage to galactose (see Fig. 1). In the case of A antigen the last sugar is N-acetylgalactosamine (GalNAc) while in the case of B antigen the last sugar is galactose (Gal).

If these two terminal sugars are eliminated from the common structure the corresponding antibodies lose their reactivity. Therefore these sugars are immunodominant within the epitope. The H antigen is the natural precursor of A and B antigen and its fucose residue is required for A and B glycosyltransferases to recognize it as the acceptor and transfer GalNAc or Gal to its terminal Gal. In the case of O individuals it rests without further elongation.

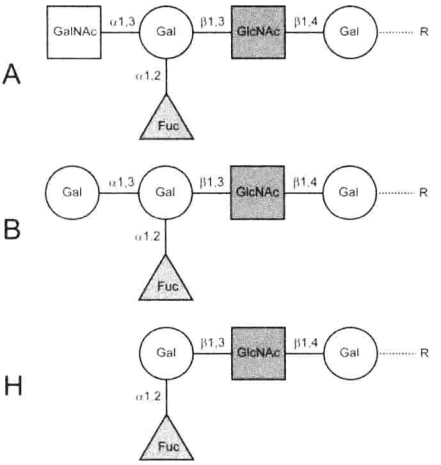

Fig. 1. ABH antigens of Type 2 core structure are schematically drawn showing their chemical composition and the nature of their glycosidic bonds. GalNAc, Gal, GlcNAc and Fuc stand for N-acetylgalactosamine, galactose, N-acetylglucosamine and fucose respectively. R represents the reducing end of the carbohydrate chain.

These antigens reside at the end of carbohydrate structures of variable length. Depending on the disaccharide precursor core chain on which ABH determinants are synthesized, they can be further divided into different types:

Type 1: Galβ1->3GlcNAcβ1-> R

Type 2: Galβ1->4GlcNAcβ1-> R

Type 3: Galβ1->3GalNAcα1-> R

Type 4: Galβ1->3GalNAcβ1-> R

Type 5: Galβ1->3Galβ1-> R

Type 6: Galβ1->4Glcβ1-> R

The internal reducing end of these precursors is bound to carrier molecules (R) of diverse nature: oligosaccharides, glycolipids or glycoproteins (Clausen & Hakomori, 1989). Types 1 through 4 are found on RBCs although Type 2 is the most common on those cells, while Type 6 is present in free oligosaccharides and some tissues (renal vein, intestinal cells) (Björk et al., 1987; Holgersson et al., 1990). Finally Type 5 is synthetic and it was utilized in the characterization of monoclonal antibodies against ABH (Oriol et al., 1990). In turn, these antigens can be present on the cell membrane bound to embedded glycoproteins or glycolipids or also forming part of these glycoconjugates but suspended in fluids as plasma or exocrine secretions and finally as free oligosaccharides without any protein or lipid carrier.

3.1 Carrier molecules

ABH substances are present on glycoproteins as terminal structures of two main types of protein modifying glycans: N-glycans and O-glycans. N-glycans are highly branched oligosaccharides attached to the amide nitrogen of asparagine through an N-acetylglucosamine residue while O-glycans, which could be simple or complex structures, are attached to the hydroxyl oxygen atom of serine or threonine residues through N-acetylgalactosamine sugar.

On RBCs, ABH antigens are present as terminal modifications of N-glycans. The most abundant glycoproteins carrying these ABH determinants are the anion exchange protein band 3, and the glucose transport protein band 4.5, as well as the urea transporter and the water channel AQP1 (aquaporin-1), which are the carrier of blood groups Kidd and Colton, respectively (Fukuda & Fukuda, 1981; Smith et al., 1994; Lucien et al., 2002). The other most abundant red cell glycoprotein, glycophorin A, on which the MNS blood group resides, does not appear to carry any ABH antigen.

Apart from glycoproteins, ABH antigens are also found as terminal modifications of glycolipids. Before 1980, it was generally considered that most of ABH determinants on RBCs were actually carried on glycosphingolipids but after that year several studies demonstrated that glycoproteins were the main carriers (Finne et al., 1980). ABH antigens on lipids are carried predominantly by glycosphingolipids. These molecules consist of a carbohydrate chain attached to ceramide, and according to the nature of the internal carbohydrate chain they are classified into six different series:

Lacto series:	Galβ1->3GlcNAcβ1->3Galβ1->4Glcβ1->Cer
Neolacto series:	Galβ1->4GlcNAcβ1->3Galβ1->4Glcβ1->Cer
Ganglio series:	Galβ1->3GalNAcβ1->4Galβ1->4Glcβ1->Cer
Isoganglio series:	Galβ1->3GalNAcβ1->3Galβ1->4Glcβ1->Cer
Globo series:	GalNAcβ1->3Galα1->4Galβ1->4Glcβ1->Cer
Isoglobo series:	GalNAcβ1->3Galα1->3Galβ1->4Glcβ1->Cer

On each of these structures ABH antigens can be added to the terminal sugar being the most common the Lacto and the Neolacto series.

Additionally, free oligosaccharides containing ABH activity are also found in milk and urine. In this case, these glycans are synthesized mostly from Type 6 precursor chain (Kobata et al., 1978; Lundblad, 1978).

3.2 Antigen distribution

ABH antigens were discovered on RBCs but are also present in many other tissues. For that reason they are also called histo-blood group antigens. In blood, apart from RBCs, platelets also present these antigens although in variable quantities depending on the individuals and their blood group. ABH antigens are detected on endothelial cells and epithelia from the lung and the gastrointestinal tract and also on the lining of the urinary and reproductive tracts. The presence of the antigens is therefore relevant for cell, tissue or organ transplantation (reviewed in (Ravn & Dabelsteen, 2000)).

4. ABO gene and the A and B antigens biosynthesis

We mentioned before that these glycan antigens are not directly encoded by genes. The A and B antigens are synthesized by enzymatic reactions catalyzed by two different enzymes called glycosyltransferases (transferases), the A transferase (α1,3-N-acetyl-D-galactosaminyltransferase) and the B transferase (α1,3-D-galactosyltransferase), respectively. Both, A and B transferases catalyze the last step on the synthesis of A and B antigen adding a GalNAc or a Gal to a precursor chain, the H antigen, by an identical α1-3 glycosidic linkage (for a review in glycosyltransferase biochemistry see (Hakomori, 1981)).

The gene is located in the long arm of chromosome 9 (9q34) and extends over more than 18 kilobases (kb). The gene has the coding sequence distributed in 7 exons, being the last one the largest. The glycosyltransferase catalytic domain is encoded in the last two exons. The 3'UTR region contains repetitive sequences that could be involved in the mRNA stability. Promoter activity resides in the gene sequence just upstream of the transcription initiation site (Yamamoto et al., 1995).

Probing cDNA libraries obtained from human adenocarcinoma cell lines of different ABO phenotypes, we successfully defined the main alleles. It was concluded that the distinct donor nucleotide-sugar specificity between A and B transferases is the result of 7 substitutions out of 1062 coding nucleotides, and only 4 of them resulting in amino acid substitutions (Arginine, Glycine, Leucine and Glycine in A transferase and Glycine, Serine, Methionine and Alanine for B transferase at codons 176, 235, 266, 268) (Yamamoto et al., 1990a).

Together with previous mutagenic studies (Yamamoto & McNeill 1996), the elucidation of the 3-D structure of the glycosyltransferases has allowed to clarify the roles of these amino acids (Patenaude et al., 2002) (see Fig. 2). The amino acid residues at codons 266 and 268 are directly involved in the recognition and binding of the sugar portion of the nucleotide-sugar donor substrates in the glycosyltransferase reaction. The amino acid residue at codon 176 is relatively far from the catalytic center, while the amino acid residue at codon 235 is at a middle distance.

The O allele encodes a non-functional glycosyltransferase enzyme. Most of O alleles contained a single nucleotide deletion at the 261 position, relatively close to the N-terminal of the coding sequence, resulting in a codon frameshift starting from amino acid 88 in the protein sequence, which causes the production of a truncated non-functional protein. This truncated protein has no catalytic domain and its mRNA transcript is less stable (O'Keefe & Dobrovic, 1996).

The antigen biosynthesis realized by the active glycosyltransferases takes place in the Golgi apparatus. These transferases are classified as type II transmembrane proteins as their structures follow the common pattern of a short transmembrane domain followed by a stem region and a catalytic domain within the Golgi lumen. The 3-D structure showed that the catalytic site is composed of two main domains. The N-terminal domain recognizes the nucleotide-sugar donor substrate. In the case of the A allele, which encodes for an α1,3-N-acetylgalactosaminyltransferase, the sugar donor is uridine diphosphate-N-acetyl-D-galactosamine (UDP-GalNAc) while the B gene product, an α1,3-galactosyltransferase transfers galactose from UDP-galactose. The C-terminal domain binds to the acceptor substrate, the fucosylated galactosyl residue of the H antigen.

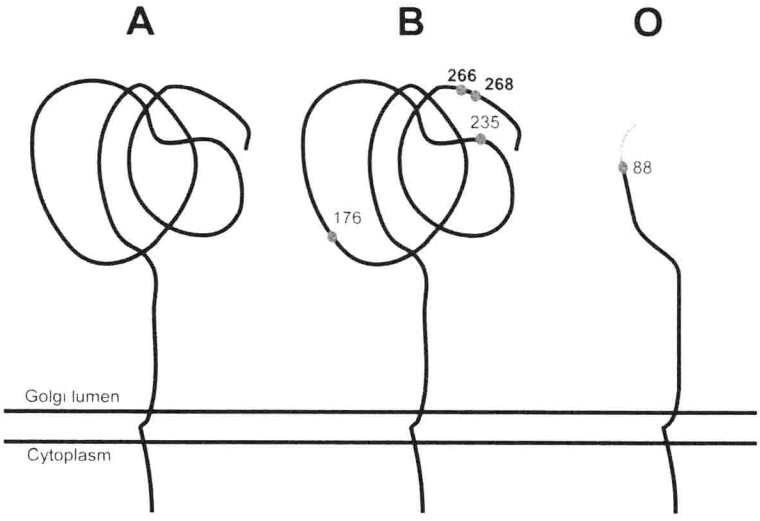

Fig. 2. Schematics with the main alleles' products and their modified residues. The different amino acids are numbered on B, in boldface the ones defining the sugar donor specificity, while in O the first amino acid after the frameshift is indicated and the alternative translation is in grey.

Since the discovery of the major alleles numerous polymorphisms/mutations have been described for the ABO gene (Yamamoto, 2004). The majority of these variations are nucleotide changes resulting in amino acid substitutions or a single nucleotide deletion/insertion and correlate well with the presence of specific subgroup phenotypes. At the moment the total number of alleles deposited in the ABO system section of the Blood Group Antigen Gene Mutation Database (dbRBC at NCBI http://www.ncbi.nlm.nih.gov/gv/mhc/xslcgi.cgi?cmd=bgmut/home) has surpassed 270 and considering the rapid advancement of next-generation sequencing, this number is going to increase in the coming years.

5. Subgroups of A and B

Many weak subgroups have been described for A group and a lower number for B group. These rare phenotypes account for a minority of individuals. Different strategies have been developed to test for these less common occurrences when the results of forward and reverse tests do not match or other inconsistencies are found (genetic for example).

The first subgroups to be recognized were A_1 and A_2. In 1910, Dungern and Hirszfeld noticed differences in the amount of A antigen expression present in A individuals (von Dungern & Hirszfeld, 1910). It was also observed that serum from B group blood presented two different antibodies reacting to A RBCs. One general anti-A that was able to react towards all A erythrocytes, and one specific to A_1. A_1 phenotype is characterized by the presence of a higher number of A antigen when compared to A_2 (Rochant et al., 1976). The A^1 allele is dominant over A^2 and encodes for an A transferase with higher affinity and reactivity for the substrates of the reaction (UDP-GalNAc and H-antigen/2'-fucosyllactose) than the A_2 transferase (Schachter et al., 1973).

Other subgroups of A include weak expressors of the antigen (A_3, A_{end}, A_{finn}, A_{bantu}, A_x, A_m, A_y, A_{el}) and the intermediate phenotype between A_1 and A_2, A_{int}. Cartron developed a method to assess the relative agglutinability of cells by radiolabelled anti-A antibodies. This quantification permitted to classify the phenotypes in respect to RBC A antigen expression and demonstrated substantial individual variation within the subgroups (Cartron et al., 1974). Further characterization includes the determination of anti-A or anti-A_1, the A transferase activity in serum, and the presence of A and/or H antigens in saliva.

The B phenotype does not present so many variants and they are more difficult to cluster in coherent groups so the weak variants have been classified by similarity to A subgroups. They are B_3, B_x, B_m, B_{el} and B_w and present various degrees of B transferase activity in plasma and secretions and weak B antigen expression.

It has to be pointed out that the phenotypic classifications do not completely correlate genetically as different weak alleles may be included in each subgroup and some have not been included in any of the existing categories (polymorphisms reviewed in (Yip, 2002)).

An interesting phenomenon has been reported, which was recognized by not adhering to classic Mendelian type of inheritance. Usually, the expression of A and B antigens is specified by two separate A and B alleles, one derived from the mother and the other one derived from the father. That is known as a common AB phenotype (*trans*-AB). However, in

unusual cases of AB phenotype the expression of both A and B antigens is apparently specified by a single gene derived from either one of the parents resulting in what it is referred to as *cis*-AB (Yamamoto et al., 1993a; Yazer et al., 2006).

A similar phenomenon named B(A) was reported when weak A reactivity was demonstrated using a monoclonal anti-A reagents on the blood of certain B-type individuals. It appears that small quantities of A antigens, in addition to larger quantities of B antigens, were produced by special B transferase. Various different alleles for both *cis*-AB and B(A) have been found (Yamamoto et al., 1993b).

The assignment to these subgroups and its confirmation requires further tests than forward and reverse typing and may include the detection of the transferase activity in serum, the detection of antigens in saliva and/or genetic confirmation.

6. H and Secretor genes and related phenotypes

A and B antigen are synthesized on the same common fucosylated precursor, the H antigen. This precursor is produced by the transfer of an L-fucose residue from guanosine diphosphate (GDP)-L-fucose to the C-2 position of the terminal galactose of Type 1 or 2 core precursor chains using an α1-2 glycosidic bond. There are two α1,2-L-fucosyltransferases that are able to catalyze this reaction, encoded by two genes, FUT1 (H) and FUT2(Se). Both produce H-active structures but their expression is tissue-dependent. On one hand, the Hh system is a blood group on its own and it is closely related to ABO. On the other hand, the product of the Secretor gene allows the ABH antigens to be present in secretions and therefore it is also of relevance. Both genes and the related phenotypes will be discussed briefly.

6.1 Hh

H-transferase, the product of FUT1 is primarily present in tissues derived from ectoderm and mesoderm and is responsible for the synthesis of RBCs, bone marrow, vascular endothelium, skin and primary sensory neurons H antigens.

FUT1 and FUT2 share about 70% sequence identity and are 35 kb apart on the long arm of chromosome 19 (19q13.3). FUT1 gene consists of 4 exons, and the catalytic region is contained in the last one. Only one transcript has been described and it is translated into a 365 amino acid long protein. In addition, FUT1 has a preferential affinity for Type 2 acceptor substrate than for Type 1.

Some FUT1 alleles producing H-deficient phenotypes have been described due to different types of mutations, mostly missense mutations, but also to deletions causing frameshifts in the coding region. Those without or reduced enzymatic activity (h alleles) are the cause of the Bombay and para-Bombay phenotypes. The Bombay phenotype is characterized by the total absence of H antigen on RBCs and secretions irrespectively of the ABO status. These individuals are typed as O by the routine ABO typing because the A or B transferases, even if present, cannot synthesize their products due to the absence of precursor. These phenotypes are very uncommon but can be locally relevant (Mollicone et al., 1995).