Università Campus Bio-Medico di Roma

Corso di dottorato di ricerca in

Scienze Biomediche Integrate e Bioetica

XXXIII ciclo a.a. 2017-2018

Determinants of severe liver disease in obese subjects: the leading role of central obesity

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INDEX

ABSTRACT

Background and aims: The obesity epidemic is closely associated with the rising prevalence and severity of nonalcoholic fatty liver disease (NAFLD): obesity has been linked not only with simple steatosis, but also with advanced liver disease, i.e. nonalcoholic steatohepatitis (NASH), NASH-related cirrhosis and hepatocellular carcinoma (HCC). A great amount of evidence on the role of genetics in NAFLD/NASH has been produced during the last 10-15 years by candidate gene and, mainly, genome-wide association studies (GWAS). Many polymorphisms have been proposed, a few of which have already acquired a recognized role in the physiopathology of NAFLD.

The reliance on measurements of body mass index (BMI) alone has proven inadequate to help clinicians assess and manage severe liver disease in their obese patients. The waist circumference (WC) revealed to be a simple method to assess abdominal adiposity, easy to standardize and to be clinically applied, and is strongly associated with severe liver disease (SLD) with or without adjustment for BMI.

Methods: The UK biobank contains data on 502.536 persons. After adoption of exclusion criteria we selected a population of 330.046 persons, from these we identified the obese population (80.224). Cox regression was performed to estimate the risk of severe liver disease and to examine risk factors in patients with obesity.

Results: Risk for severe liver disease was increased in patients with obesity compared to general population. BMI alone is not sufficient, waist circumference is a simple measure of abdominal adiposity, easy to standardize and to be clinically applied. For

any given BMI the variation in waist circumference is considerable and in any given BMI category, adults with higher waist circumference values are at increased risk of severe liver disease compared with those with a lower waist circumference.

Conclusions: Combination of BMI and WC can identify the phenotype of obesity at highest risk to develop SLD, far better than either measure alone. WC should be routinely measured in clinical practice, as it can provide additional information to guide patient management.

INTRODUCTION

Liver cirrhosis is the most common cause of liver-related death and the most important risk factor for hepatocellular cancer (HCC) 1. Chronic insults to the liver eventually lead to cirrhosis, the best documented of them being alcohol abuse as well as hepatitis B and C. Non-alcoholic fatty liver disease (NAFLD) has emerged as another important risk factor for cirrhosis of the liver 2.

The term NAFLD covers a spectrum of liver diseases ranging from simple steatosis to liver cirrhosis. Steatosis was earlier considered to be a benign disorder without risk for liver-related morbidity and mortality. During the last decades, it has become evident that steatosis can progress towards steatohepatitis and end-stage liver disease 3–5. It has been proposed that an important number of cases of cryptogenic liver cirrhosis are caused by underlying NAFLD 6,7, and that NAFLD may account for a substantial number of HCCs 8,9. As known, the risk for severe liver disease is increased in patients with obesity compared to general population 10. Overweight, including obesity, is a major health problem worldwide 11–13, with well-known negative effects on cardiovascular health. Obesity is usually defined as elevated body mass index (BMI). Although being practical, the consideration of total body mass completely ignores possible variation in body composition. Measures of central or visceral adiposity are closely linked to development of metabolic and cardiovascular complications 14,15, while peripheral fat may exert a neutral or even protective effect 16.

Waist circumference reflects the proportion of body fat more accurately than BMI does. Central (abdominal) obesity, as assessed by waist circumference, is a fundamental component of fully expressed metabolic syndrome and may play a major role in its early development 10.

BMI was determined as body weight/body height (kg/m²). Waist circumference was measured in the standing position, at the middle point between the anterior iliac crest and the lower border of the ribs. Waist circumference ≥ 90 cm in males and ≥ 85 cm in females was considered as risky waist.

According to the available data, heritability estimates for hepatic fat content range from 20% to 70%, and an almost 80% of shared heritability has been found between hepatic fat content and fibrosis. The rs738409 single nucleotide polymorphism (SNP) in patatin-like phospholipase domain-containing protein 3 gene and the rs58542926 SNP in transmembrane 6 superfamily member 2 gene have been robustly associated with NAFLD and with its progression 17.

Regarding liver diseases, being overweight or obese is associated with more advanced stages of NAFLD and faster progression of chronic liver diseases, like hepatitis C, primary biliary cirrhosis and alcoholic liver disease 18–21.

Nevertheless, BMI has been criticized for misclassification bias, mainly classifying persons with high muscular mass as overweight or obese and it is known that visceral adiposity is more relevant than subcutaneous fat deposits in the development of NAFLD.

If measures better reflecting visceral adiposity are superior to BMI at predicting the future risk of severe liver disease has so far not been investigated. A number of body composition measures have been associated with mortality independent of BMI, and include known and more unknown measures such as waist circumference, waist-hip ratio (WHR), body fat percentage using bioelectric impedance, waist-to-height ratio (WHtR), waist-to-hip-to-height 1 ratio (WHHR), and A Body Shape Index (ABSI) [22-25].

CHAPTER 1: ORGANOGENESIS AND DEVELOPMENT OF THE LIVER

LIVER DEVELOPMENT

The liver is the largest internal organ providing essential metabolic, exocrine and endocrine functions. These include production of bile, metabolism of dietary compounds, detoxification, regulation of glucose levels through glycogen storage and control of blood homeostasis by secretion of clotting factors and serum proteins such as Albumin. Hepatocytes are the principal cell type in the liver accounting for ~70% of the mass of the adult organ. Hepatocytes, along with biliary epithelial cells (BECs; also known as cholangyocytes) are derived from the embryonic endoderm, while the stromal cells, stellate cells, kuppfer cells and blood vessels, are of mesodermal origin (see Fig. 1). The use of animal models, such as the mouse, chicken, zebrafish and Xenopus, as well as primary cell cultures has identified many of the genes and molecular pathways regulating embryonic liver development. These studies show that much of hepatogenesis is evolutionarily conserved and occurs through a progressive series of reciprocal tissue interactions between the embryonic endoderms and nearby mesoderm (see Fig. 2) 26,27. The application of this information has recently enabled researchers to produce "hepatic-like" tissue from embryonic stem (ES) cells in vitro, which may ultimately lead to therapeutically useful tissue for transplantation. This review summarizes the current understanding of liver and biliary system development focusing on studies the in mouse embryo where this process is best understood.

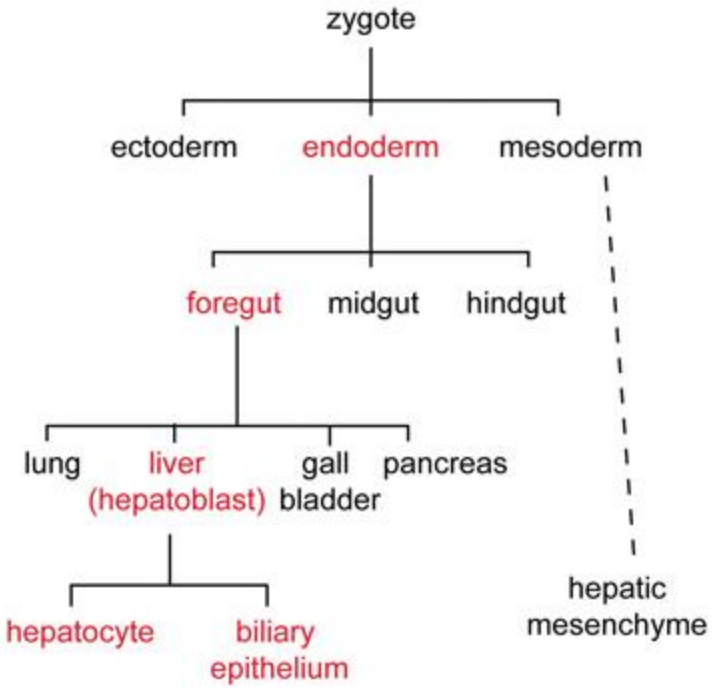


Figure 1. Liver cell lineage. The cell lineage steps during hepatic development (red) from uncomitted endoderm to functional adult hepatocytes and biliary epithelium

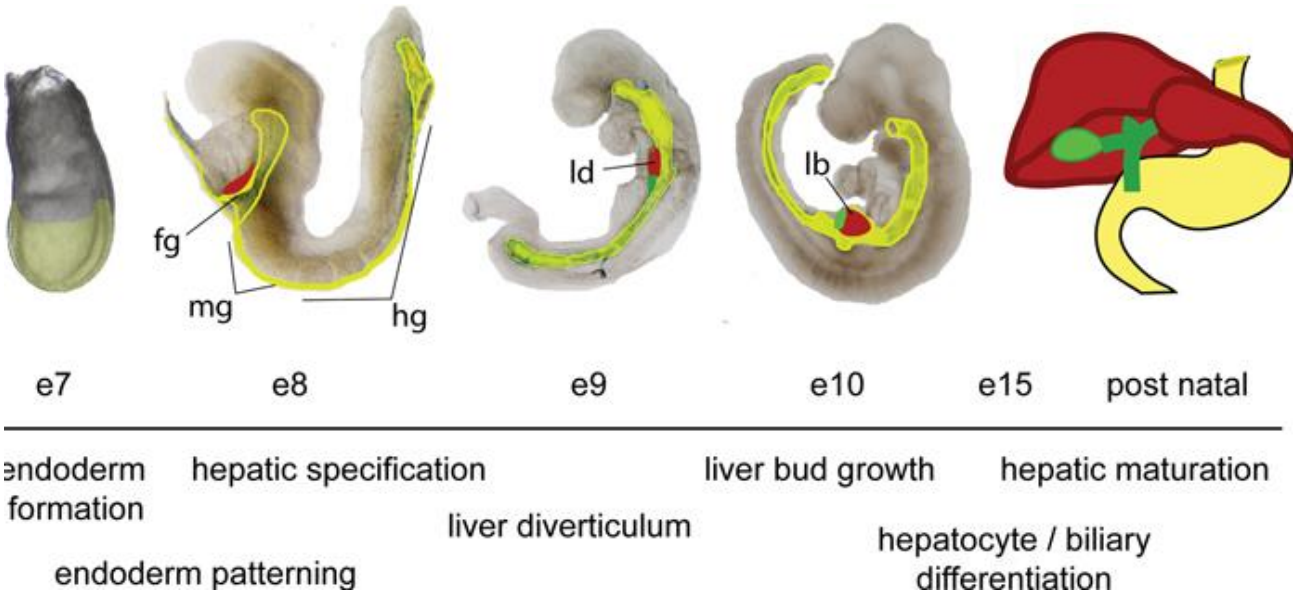


Figure 2. Time line of mouse liver development. The schematic shows mouse embryos at different stages of development with the endoderm tissue highlighted in yellow, the liver in red and the gall bladder in green.

Overview of liver development

The endoderm germ layer is established during gastrulation and forms a primitive gut tube that is subdivided into foregut, midgut and hindgut regions (see Fig. 2). Fate mapping studies in the mouse embryo at embryonic day 8.0 of gestation (e8.0) indicate that the embryonic liver originates from the ventral foregut endoderm 28.

The first morphological sign of the embryonic liver is the formation of the hepatic diverticulum, an out-pocket of thickened ventral foregut epithelium adjacent to the developing heart at e9.0 (see Fig. 2). The anterior portion of the hepatic diverticulum gives rise to the liver and intrahepatic biliary tree, while the posterior portion forms the gall bladder and extrahepatic bile ducts. At e9.5, the hepatic endoderm cells, known as hepatoblasts delaminate from the epithelium and invade the adjacent septum transversum mesenchyme (STM) to form the liver bud 29-31.

The STM contributes fibroblasts and stellate cells of the liver. Between e10–15 the liver bud undergoes a period of accelerated growth as it is vascularized and colonized by hematopoietic cells to become the major fetal hematopoietic organ.

The hepatoblasts are bi-potential and those residing next to the portal veins become BECs that will line the lumen of the intrahepatic bile ducts (IHBD), while the majority of hepatoblasts in the parenchyma differentiate into hepatocytes. The maturation of functional hepatocytes and the formation of a biliary network connected to the

extrahepatic bile ducts (EHBD) are gradual. Beginning at e13 this process continues until after birth to generate the characteristic tissue architecture of the liver.

Cellular architecture of the liver

Within the adult liver, the IHBD, portal vein and hepatic artery run in parallel and are referred to as the "portal triad" (see Fig. 3). The portal triad is surrounded by hepatocytes arranged in single cell sheets known as hepatic plates, separated by sinusoid spaces that are connected to a network of blood vessels capillaries. Blood plasma from the portal vein enters the sinusoid space and comes into direct contact with the basal surface of hepatocytes, which absorb metabolites and toxins. Bile is secreted from the apical surface of adjoining hepatocytes into the bile canaliculi (grooves in the cell surface), and then flows though the IHBD to the extrahepatic bile ducts (EHBD), and into the gall bladder where it is stored before release into the duodenum. This cellular architecture is essential for proper hepatic function.

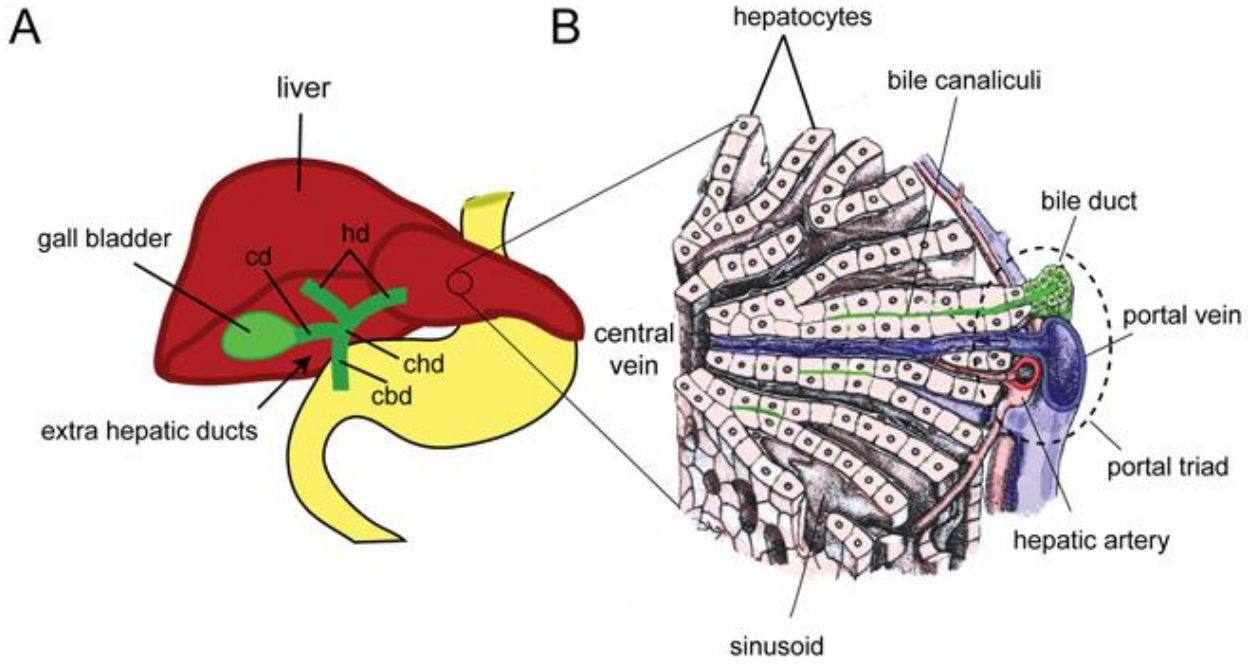


Figure 3. Cellular architecture of the liver. (**A**) The schematic shows an adult liver (red), with the gall bladder and extra hepatic ducts (green), in relation to the stomach and intestine (yellow). The extra hepatic duct system consists of the hepatic ducts (hd), which drain bile from the liver into the common hepatic duct (chd) to the gall bladder via the cystic duct (cd) and into the duodenum through the common bile duct (cbd). (**B**) A schematic of the cellular architecture of the liver showing the hepatocytes (pink) arranged in hepatic plates separated by sinusoid spaces radiating around a central vein. Bile canaliculi on the surface of adjoining hepatocytes drain bile into the bile ducts (green), which run parallel to portal veins (blue) and hepatic arteries (red) to form the "portal triad". (Panel B is adapted with permission from Bloom and Fawcett: A Text Book of Histology 10th Edition).

Differentiation of hepatocytes and biliary epithelial cells

The differentiation of bi-potential hepatoblasts into hepatocytes or BECs begins around e13 of mouse development. Initially hepatoblasts express genes associated with both adult hepatocytes (Hnf4α, Albumin) and BECs (cytokeratin-19), as well as fetal liver genes such as α-fetoprotein (Afp). Hepatoblasts in contact with the portal vein form a monolayer, and then a bi-layer, of cuboidal biliary precursors that increase cytokeratin-19 (CK-19) expression and down-regulate hepatic genes. Between e17 and into the perinatal period focal dilations appear in the bi-layer and these become surrounded by portal mesenchyme to form IHBD, while the remaining bi-layer cells regress (see Fig. 4). This process, which involves tubulogenesis and apoptosis, is known as ductal plate remodeling [32-33].

Hepatoblasts in the liver parenchyma that are not in contact with portal veins gradually differentiate into mature hepatocytes. At e17 hepatocytes acquire their characteristic epithelial morphology arranged in hepatic chords with bile canaliculi on the apical surfaces. While defects in early liver bud growth are often embryonic lethal, disruption in hepatocyte maturation/function or ductal plate remodeling malformations (see Fig. 5) are observed in many human disorders [34-36].

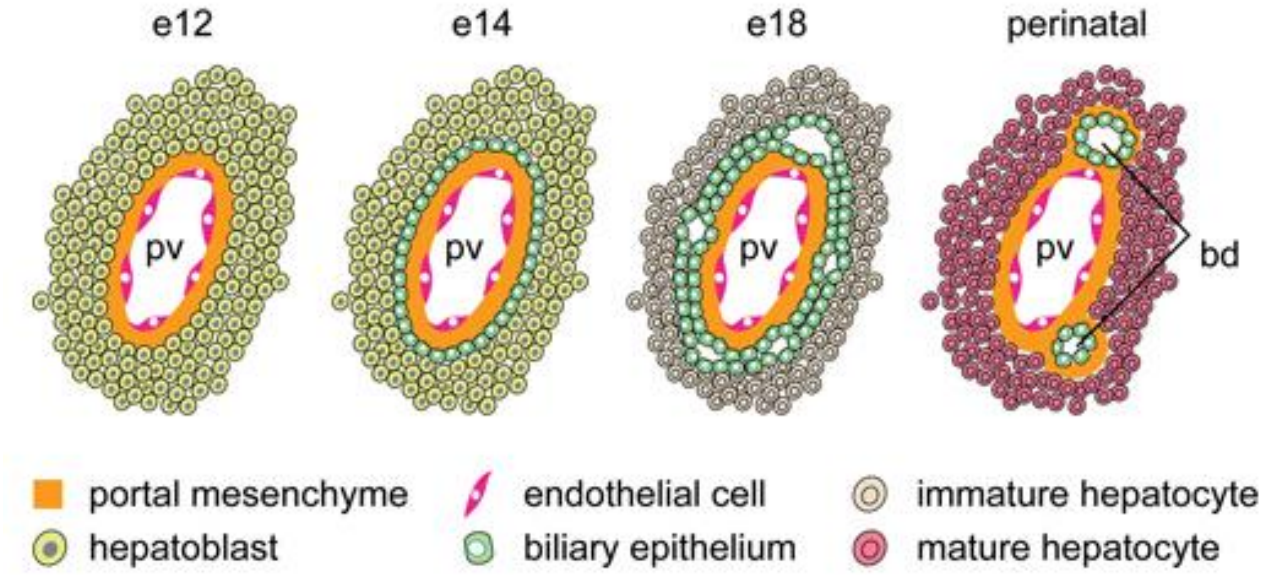


Figure 4. Bile duct formation. The schematics show the steps of intrahepatic bile duct formation. Starting at e13 hepatoblasts in contact with the portal vein mesenchyme begin to adopt a biliary epithelium fate and form cuboidal epithelail layer. This layer duplicates and from e17 to the perinatal period, a process known as ductal plate remodeling results in focal dilations in the bi-layer which become surrounded by portal mesenchyme to form bile ducts, while the remaining bi-layer cells regress. Hepatoblasts in the parenchyma differentiate into hepatocytes.

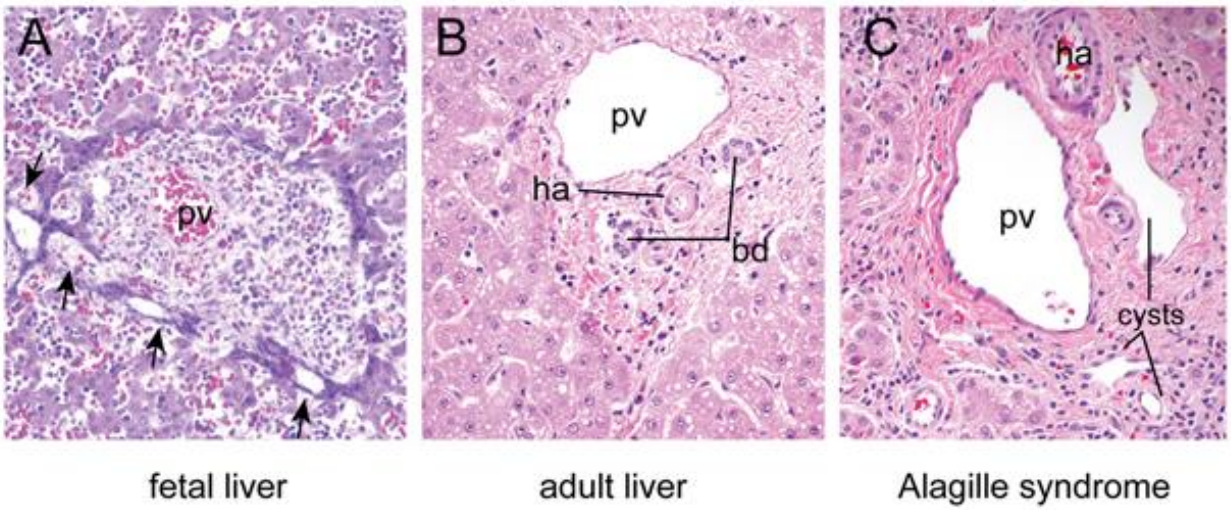


Figure 5. Histology of normal and defective intrahepatic bile ducts. Histological staining of human liver sections at: (**A**) 16 weeks of development showing focal dilations (arrows) in the bi-layer of the biliary epithelial cell precursors surrounding the portal vein (pv). (**B**) A mature liver showing the bile ducts (bd) and hepatic artery (ha) embedded in the periportal mesenchyme in a characteristic arrangement known as the "portal triad". Hepatocytes are arranged in chords surround the portal triad. (**C**) The portal triad region from an Alagille syndrome patient showing ductal cysts rather than normal bile ducts as a result of defects in ductal plate remodeling. Images courtesy of Gail Deutsch.

Hepatocyte differentiation

During mid-gestation the haematopoietic cells in the liver secrete the cytokine Oncostatin M (OSM), which in combination with glucocorticoid hormones, HGF and Wnt promotes hepatocyte differentiation 37-39.

OSM induces metabolic maturation by activating the gp130 receptor and a JAK/Stat3 signaling pathway 40, while promoting morphological maturation into polarized epithelium via K-ras and E-cadherin 41.

Some evidence suggests that HGF and OSM activity is balanced by TNFα, which inhibits maturation and maintains the proliferative capacity of fetal hepatocytes, allowing the liver to grow to the appropriate size before differentiating 42.

These secreted factors act in part by regulating a number of liver-enriched transcription factors including C/EBPα, HNF1α, Foxa1–3, nuclear hormone receptors and HNF4α (see Fig. 6), which function in a complex inter-regulatory network to control hepatocyte gene expression 43,44.

Genetic analysis has confirmed a role for HNF4α, C/EBPα and HNF1α in hepatocyte differentiation. HNF4α is first expressed in hepatoblasts at e9.0 and Hnf4α-/- fetal hepatocytes fail to express many mature hepatic enzymes and lack normal hepatocyte morphology 45,46.

Genome scale chromatin immunoprecipitation assays suggest that HNF4α binds to the promoters of nearly half of the genes expressed in the mouse liver, including genes encoding cell adhesion and junctional proteins important in hepatocyte epithelial structure 47,48.

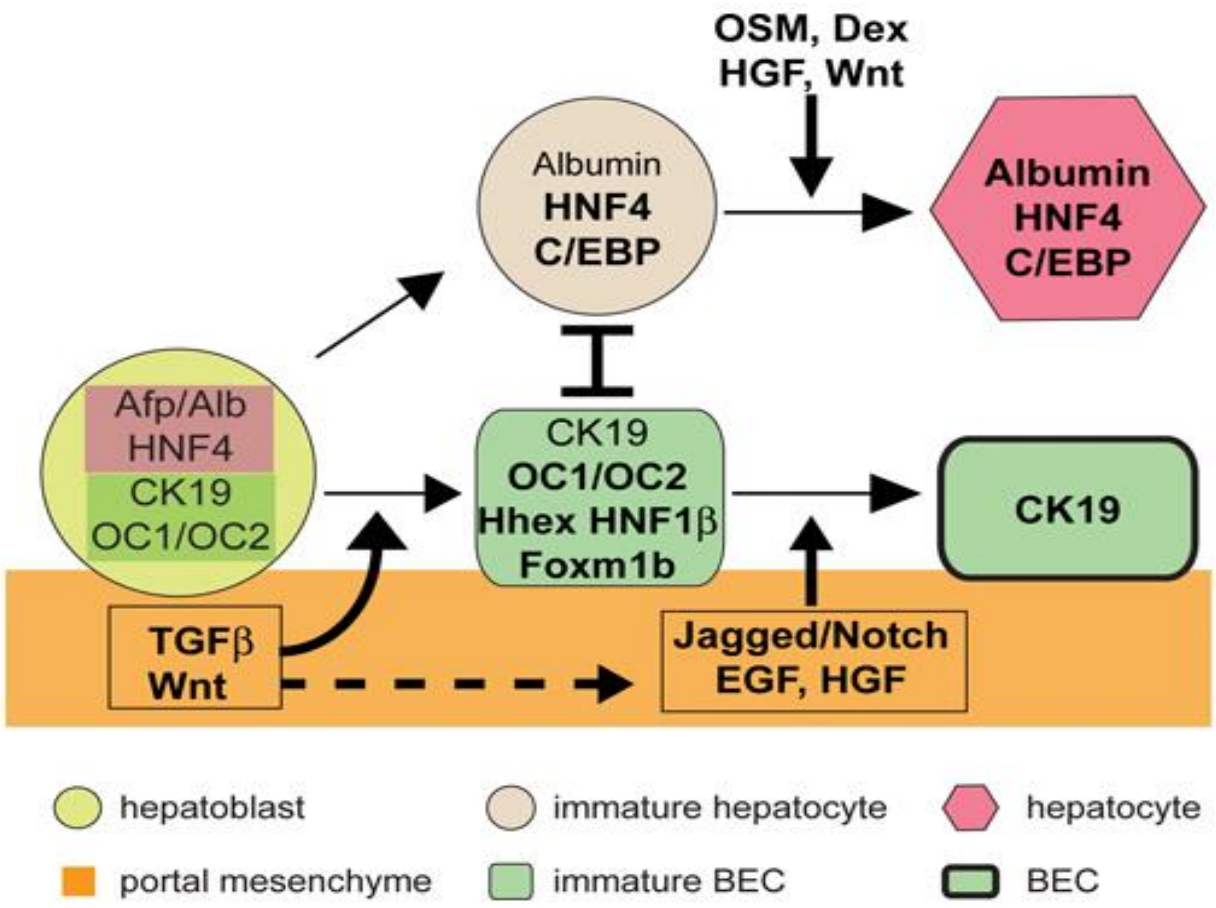


Figure 6. Hepatocyte and Biliary epithelium lineage segregation. The schematic illustrates a model of hepatoblast differentiation into hepatocytes or biliary epithelial cells (BEC). Bi-potential hepatoblasts express fetal liver genes (Afp) as well as markers of both hepatocytes (Albumin) and BECs (CK19). Evidence suggests that signals (possibly TGFβ and Wnts) from the periportal mesenchyme enhance the expression of BEC promoting transcription factors (OC1, OC2, HNF1β) in the adjacent hepatoblasts, while at the same time these signals repress the expression of hepatogenic transcription factors (HNF4 and C/EBP). In contrast hepatoblasts in the parenchyma (that do not

experience the periportal mesenchyme signals) up regulate the expression of hepatogenic factors. Mutual antagonism between the two groups of transcription factors is thought to reinforce this lineage segregation. Continued signaling (Notch, EGF and HGF) from the periportal mesnechyme are essential for ductal plate remodeling, while other factors (OSM, Dex, HGF and Wnt) promote hepatocyte maturation.

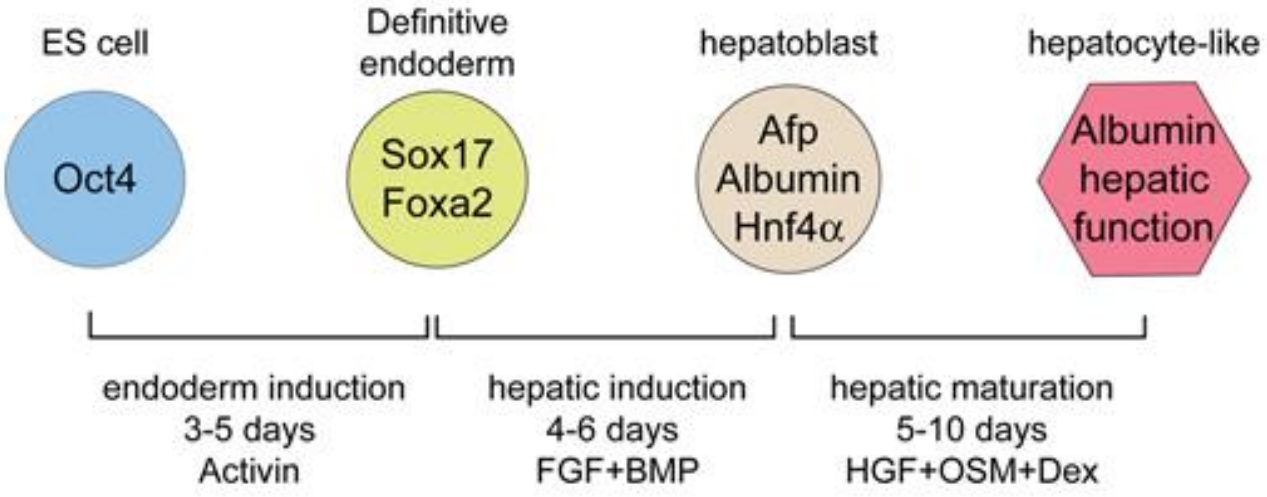


Figure 6. In vitro hepatic differentiation from ES cells. The diagram depicts a generalized protocol summarized from the work of several labs that have applied developmental paradigms to mouse and human ES cells to direct the differentiation of hepatic-like cell in vitro. The factors added to the cultures, the durations of exposure, and the developmental step that these treatments are meant to mimic are indicated below. The cell types and key lineage specific marker genes expressed in those cells are indicated.

Biliary epithelial cells

TGFβ, Wnt and Notch are candidate signals from periportal mesenchyme that promotes BEC development (see Fig. 12). There is evidence that a TGFβ signaling gradient emanating from the portal region promotes biliary differentiation in the adjacent hepatoblasts 49,50.

Wnt/β-catenin signaling also promotes BEC development 51-53, and may act in part by stimulating the expression of EGF, which along with HGF can induce the formation of biliary structures in cultured 54,55.

Human Alagille syndrome (OMIN #118450) patients with autosomal dominant mutations in the Notch ligand gene Jagged-1 have a paucity of IHBD (see Fig. 11), as do mice with compound heterozygous Jagged-1; Notch-2 mutations 56. In response to the mesenchyme signals the periportal hepatoblasts down regulate pro-hepatic factors HNF4α, Tbx3 and C/EBP (which repress BEC development) and increase expression of BEC transcription factors OC1/HNF6, OC2 and HNF1β (see Fig. 12) 57, 58.

CHAPTER 2: ANATOMY OF THE LIVER

GENERAL ANATOMY

The liver is the largest organ, accounting for approximately 2% to 3% of average body weight. The liver has 2 lobes typically described in two ways, by morphologic anatomy and by functional anatomy (as illustrated in Fig. 1).[59]

Located in the right upper quadrant of the abdominal cavity beneath the right hemidiaphragm, it is protected by the rib cage and maintains its position through peritoneal reflections, referred to as ligamentous attachments (Fig. 2). Although not true ligaments, these attachments are avascular and are in continuity with the Glisson capsule or the equivalent of the visceral peritoneum of the liver. [60] Ligamentous Attachments The falciform ligament is an attachment arising at or near the umbilicus and continues onto the anterior aspect of the liver in continuity with the umbilical fissure. The falciform ligament courses cranially along the anterior surface of the liver, blending into the hepatic peritoneal covering coursing posterosuperiorly to become the anterior portion of the left and right coronary ligaments. Of surgical importance, at the base of the falciform ligament along the liver, the hepatic veins drain into the inferior vena cava (IVC). [61]

A common misconception associated with the falciform ligament is that it divides the liver into left and right lobes. Based on morphologic anatomy, this may be true; however, this does not hold true from a functional standpoint (discussed later). Within

the lower edge of the falciform ligament is the ligamentum teres (round ligament), a remnant of the obliterated umbilical vein (ductus venosus) that travels from the umbilicus into the umbilical fissure where it is in continuity with the ligamentum venosum as it joins the left branch of the portal vein. The ligamentum venosum lies within a fissure on the inferior surface of the liver between the caudate lobe posteriorly and the left lobe anteriorly, where it is also invested by the peritoneal folds of the lesser omentum (gastrohepatic ligament). During fetal life, the ductus venosus is responsible for shunting a majority of blood flow of the umbilical vein directly into the IVC, transporting oxygenated blood from the placenta to the fetus. After birth, the umbilical vein closes as the physiologic neonatal circulation begins. In the presence of portal hypertension, the umbilical vein may recanalize to allow portasystemic collateralization through the abdominal wall, known as caput medusae. At the cranial aspect of the liver is a convex area along the diaphragmatic surface that is devoid of any ligamentous attachments or peritoneum. This bare area of the liver is attached to the diaphragm by flimsy fibroareolar tissue. The coronary ligament lies anterior and posterior to the bare area of the liver comprised of peritoneal reflections of the diaphragm. These areas converge to the left and right of the liver to form the left and right triangular ligaments, respectively. The right coronary and right triangular ligaments course posterior and caudally toward the right kidney, attaching the liver to the retroperitoneum. All attachments help fixate the liver within the right upper quadrant of the abdomen. During hepatic surgery, mobilization of the liver requires division of these avascular attachments. In upper abdominal surgery, the liver has close associations with many

structures and organs. The IVC maintains an intimate relationship to the caudate lobe and right hepatic lobe by IVC ligaments.62

These caval ligaments are bridges of broad membranous tissue that are extensions of the Glisson capsule from the caudate and right hepatic lobe. Of surgical importance, these ligaments are not simple connective tissue but rather contain components of hepatic parenchyma, including the portal triads and hepatocytes. Hence, during liver mobilization, these ligaments must be controlled in a surgical manner to avoid unnecessary bleeding or bile leakage during hepatic surgery. Perihepatic Organs/Anatomy The gastrointestinal tract has several associations with the liver (illustrated in Fig. 3). The stomach is related to the left hepatic lobe by way of the gastrohepatic ligament or superior aspect of the lesser omentum, which is an attachment of connective tissue between the lesser curvature of the stomach and the left hepatic lobe at the ligamentum venosum. Important neural and vascular structures may run within the gastrohepatic ligament, including the hepatic division of the vagus nerve and, when present, an aberrant left hepatic artery as it courses from its left gastric artery origin. The hepatic flexure of the colon where the ascending colon transitions to the transverse colon is in close proximity or sometimes in direct contact with the right hepatic lobe. Additionally, the duodenum and portal structures are in direct association with the liver through the hepatoduodenal ligament (inferior aspect of the lesser omentum) and porta hepatis. Anatomic understanding of the portal anatomy is essential to hepatic resection and associated vascular and biliary reconstructions. Within the porta hepatis is the common bile duct, hepatic artery, and portal vein that course in a lateral,

medial, and posterior configuration, respectively. The foramen of Winslow (epiploic foramen) has important relevance to the porta hepatis and hepato-pancreatico-biliary surgery. The foramen of Winslow, originally described by the Danish anatomist Jacob Winslow in 1732, is a communication or connection between the abdominal cavity and the lesser sac. During hepatic resection, need for complete control of the hepatic vascular inflow may be accomplished by a Pringle maneuver. 63, 64 This maneuver, developed by an Australian surgeon, James Hogarth Pringle, while in Glasgow, Scotland, during the management of hepatic trauma, involves occlusion of the hepatic artery and portal vein inflow through control of the porta hepatis. This may be done by placement of a large clamp on the porta hepatis or more atraumatically with the use of a tourniquet passed through the foramen of Winslow and pars flaccida (transparent portion of lesser omentum overlying caudate lobe) encircling the porta hepatis. The gallbladder resides in the gallbladder fossa at the posterior interface of segments IV and V. It establishes continuity with the common bile duct via the cystic duct. Additionally, the cystic artery most commonly arises as a branch off the right hepatic artery. Understanding of portal vasculature and biliary anatomy is crucial given its wide anatomic variability to avoid inadvertent injury during any hepatic, pancreatic, biliary, or foregut surgery. Additionally, the right adrenal gland lies within the retroperitoneum under the right hepatic lobe. The right adrenal vein drains directly into the IVC; hence, care should be taken during hepatic mobilization so as to avoid avulsion of the vein or inadvertent dissection into the adrenal gland as this can result in significant hemorrhage.

LYMPHATIC AND NEURAL NETWORK

The liver possesses a superficial and deep lymphatic network through which lymph produced in the liver drains.65 The deep network is responsible for greater lymphatic drainage toward lateral phrenic nodes via the hepatic veins and toward the hilum through portal vein branches. The superficial network is located within the Glisson capsule with an anterior and posterior surface. The anterior surface primarily drains to phrenic lymph nodes via the bare area of the liver to join the mediastinal and internal mammary lymphatic networks. The posterior surface network drains to hilar lymph nodes, including the cystic duct, common bile duct, hepatic artery, and peripancreatic as well as pericardial and celiac lymph nodes. The lymphatic drainage patterns have surgical implications with regard to lymphadenectomy undertaken for cancer of the gallbladder, liver, and pancreas.

The neural innervation and controls of liver function are complex and not well understood. However, like the remainder of the body, the liver does have parasympathetic and sympathetic neural innervation. Nerve fibers are derived from the celiac plexus, lower thoracic ganglia, right phrenic nerve, and the vagi. The vagus nerves divide into an anterior (left) and posterior (right) branch as they course from the thorax into the abdomen. The anterior vagus divides into a cephalic and a hepatic division of which the latter courses through the lesser omentum (gastrohepatic ligament) to innervate the liver and is responsible for the parasympathetic innervation. Sympathetic innervation arises predominantly from the celiac plexus as well as the thoracic splanchnic nerves.

HEPATIC VASCULATURE

The liver is a very vascular organ and at rest receives up to 25% of total cardiac output, more than any other organ. Its dual blood supply is uniquely divided between the hepatic artery, which contributes 25% to 30% of the blood supply, and the portal vein, which is responsible for the remaining 70% to 75%. The arterial and portal blood ultimately mixes within the hepatic sinusoids before draining into the systemic circulation via the hepatic venous system.66

Arterial Vasculature Although the arterial vasculature of the liver is variable, the most common configurations are discussed in this article. As illustrated in Fig. 4, in the most common arterial configuration, the common hepatic artery originates from the celiac axis along with the left gastric and splenic arteries. The common hepatic artery proceeds laterally and branches into the proper hepatic artery and the gastroduodenal artery. The gastroduodenal artery proceeds caudally to supply the pylorus and proximal duodenum and has several indirect branches to the pancreas. The proper hepatic artery courses within the medial aspect of the hepatoduodenal ligament and porta hepatis toward the liver to divide into left and right hepatic arteries to feed the respective hepatic lobes. Additionally, the right gastric artery has a variable origin arising from the hepatic artery as it courses laterally. The cystic artery to the gallbladder commonly arises from the right hepatic artery. In Fig. 5, common arterial variants are illustrated. The most common variants include aberrant (replaced) hepatic arteries in which the dominant hepatic arteries do not arise from the proper hepatic artery but rather from an alternate

origin. An aberrant left hepatic artery typically arises from the left gastric artery and courses through the lesser omentum to supply the left liver and is seen in approximately 15% of patients. In spite of its alternate origin, the aberrant left hepatic artery still enters the liver through the base of the umbilical fissure in a medial orientation, similar to that of a native left hepatic artery. An aberrant right hepatic artery, seen in approximately 20% of patients, most commonly arises from the superior mesenteric artery. Unlike its left hepatic artery counterpart, the aberrant right hepatic artery often courses posterolateral in the hepatoduodenal ligament to enter the right liver. Venous Vasculature The portal vein provides the bulk of the nutritive blood supply to the liver. As illustrated in Fig. 6, the portal vein forms from the confluence of the superior mesenteric vein and splenic vein behind the neck of the pancreas. Additional venous branches that drain into the portal vein include the coronary (left gastric) vein, cystic vein, and tributaries of the right gastric and pancreaticoduodenal veins. The portal vein is valveless and is a low-pressure system with pressures typically 3 to 5 mm Hg. The coronary (left gastric) vein is of particular importance clinically as it becomes a major portasystemic shunt in the face of portal hypertension and feeds the gastroesophageal variceal complex. The main portal vein courses cranially toward the liver as the most posterior structure within the hepatoduodenal ligament to divide into the left and right portal veins near the liver hilum. A small branch to the right side of the caudate is commonly encountered just before or after the main portal vein branching. The left portal vein has two portions, an initial transverse portion and then an umbilical portion as it approaches the umbilical fissure. The left portal vein tends to have a longer

extrahepatic course and commonly gives off a branch to the caudate lobe, but the caudate portal vein inflow is variable and may arise from the main or right portal vein also. The transverse portion of the left portal vein approaches the umbilical fissure and takes an abrupt turn toward it to form the umbilical portion as it enters the liver. Within the liver, the umbilical portion of the left portal vein commonly first gives off a branch to segment II before then dividing into branches to segment III and to segment IVa/IVb. The right portal vein often emerges closer to or within the hepatic parenchyma of the right liver itself. It quickly divides into anterior and posterior branches to segments V and VIII and segments VI and VII, respectively (see Fig. 1; Figs. 7 and 8). The venous drainage of the liver is through the intrahepatic veins that ultimately coalesce into three hepatic veins that drain into the IVC superiorly. The left and middle hepatic veins may drain directly into the IVC but more commonly form a short common trunk before draining into the IVC. The right hepatic vein is typically larger, with a short extrahepatic course and drains directly into the IVC. Additional drainage occurs directly into the IVC via short retrohepatic veins and, on occasion, an inferior right accessory hepatic vein. The hepatic veins within the parenchyma are unique in that, unlike the portal venous system, they lack the fibrous, protective, encasing the Glisson capsule.[67] Ultrasonography facilitates intraoperative mapping of the internal anatomy of the liver. As seen in Fig. 9, by ultrasound, the portal venous anatomy can readily be identified by the echogenic, hyperechoic Glisson capsule surrounding the portal veins, whereas the hepatic veins lack this. The IVC maintains an important and intimate association with the liver as it courses in a cranial-caudal direction to the right of the aorta. As the IVC

travels cranially, it courses posterior to the duodenum, pancreas, porta hepatis, caudate lobe, and posterior surface of the liver as it approaches the bare area where it receives the hepatic venous outflow from the hepatic veins. Multiple small retrohepatic veins enter the IVC along its course, mostly from the right hepatic lobe. Hence, in mobilizing the liver or during major hepatic resections, it is imperative to maintain awareness of the IVC and its vascular tributaries at all times.

BILIARY TREE

The intrahepatic biliary tree is comprised of multiple ducts that are responsible for the formation and transport of bile from the liver to the duodenum and typically follows the portal venous system. The right hepatic duct forms from an anterior sectoral duct from segments V and VIII and a posterior sectoral duct from segments VI and VII. The anterior sectoral duct courses in an anterior, vertical manner whereas the posterior duct proceeds in a lateral, horizontal manner. The right duct typically has a short extrahepatic course with some branching variability. Surgeons should be mindful of this variable anatomy when operating at the hilum of the liver. The left hepatic duct drains the left liver and has a less variable course as it parallels the left portal vein with a longer extrahepatic course. The left and right hepatic ducts join near the hilar plate to form the common hepatic duct. As the common hepatic duct courses caudally, it is joined by the cystic duct to form the common bile duct. The common bile duct proceeds within the lateral aspect of the hepatoduodenal ligament toward the head of the pancreas to drain into the duodenum through the ampulla of Vater. Biliary drainage of the caudate lobe is variable with drainage seen through left and right hepatic ducts in approximately 70%

to 80% of cases.8 In 15%, caudate drainage is seen through the left hepatic duct alone and the remaining 5% to 10% of cases drains through the right hepatic duct system alone. Hence, as discussed previously, surgical intervention involving the caudate lobe requires attention to biliary anatomy as well as vascular anatomy.

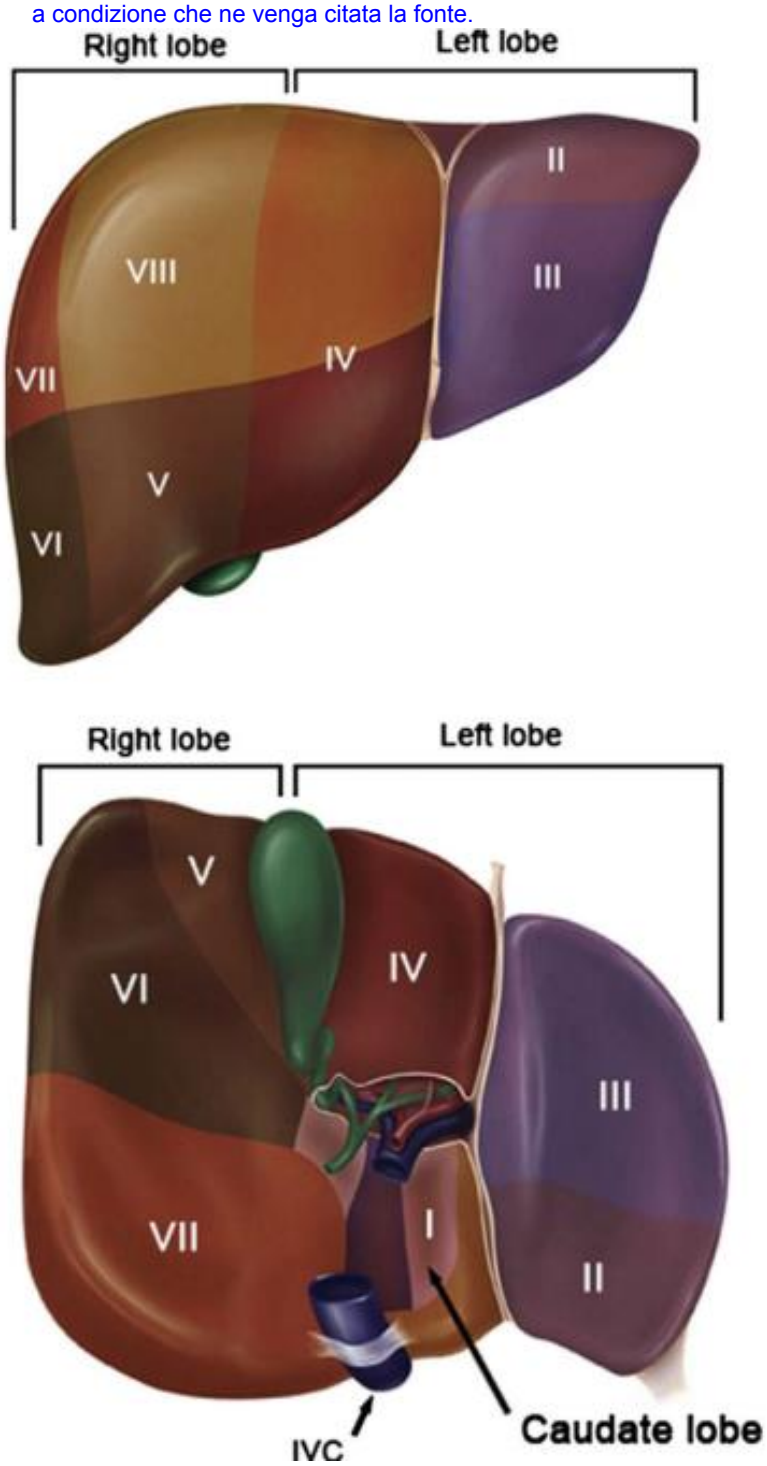


Figure 1. Anterior and posterior surfaces of liver illustrating functional division of the liver into left and right hepatic lobes with Couinaud's segmental classification based on

functional anatomy. *From* Brunicardi FC, Andersen DK, Billiar TR, et al. Schwartz's principles of surgery. 9th edition. New York: McGraw-Hill Publishing; 2010. p. 31–3; with permission.

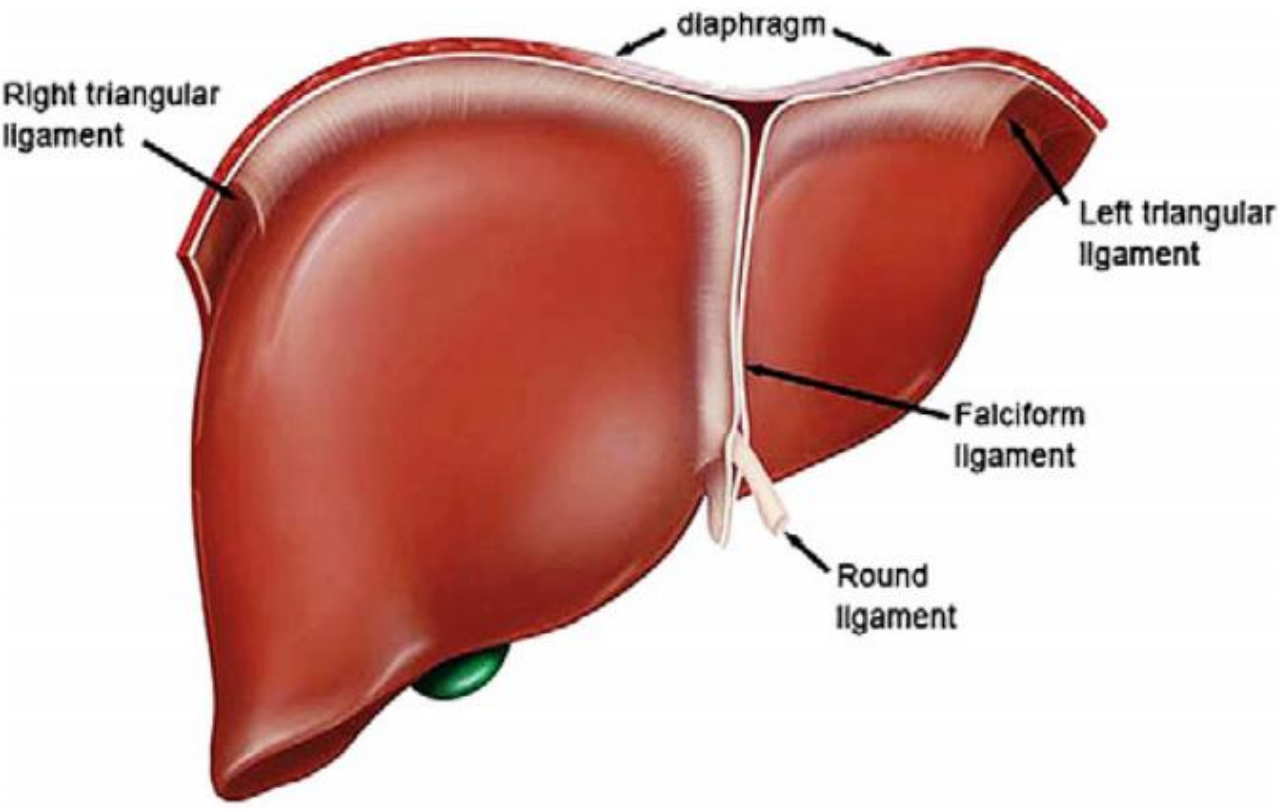


Figure 2. Ligamentous attachments of the liver. *From* Brunicardi FC, Andersen DK, Billiar TR, et al. Schwartz's principles of surgery. 9th edition. New York: McGraw-Hill Publishing; 2010. p. 31–2; with permission.

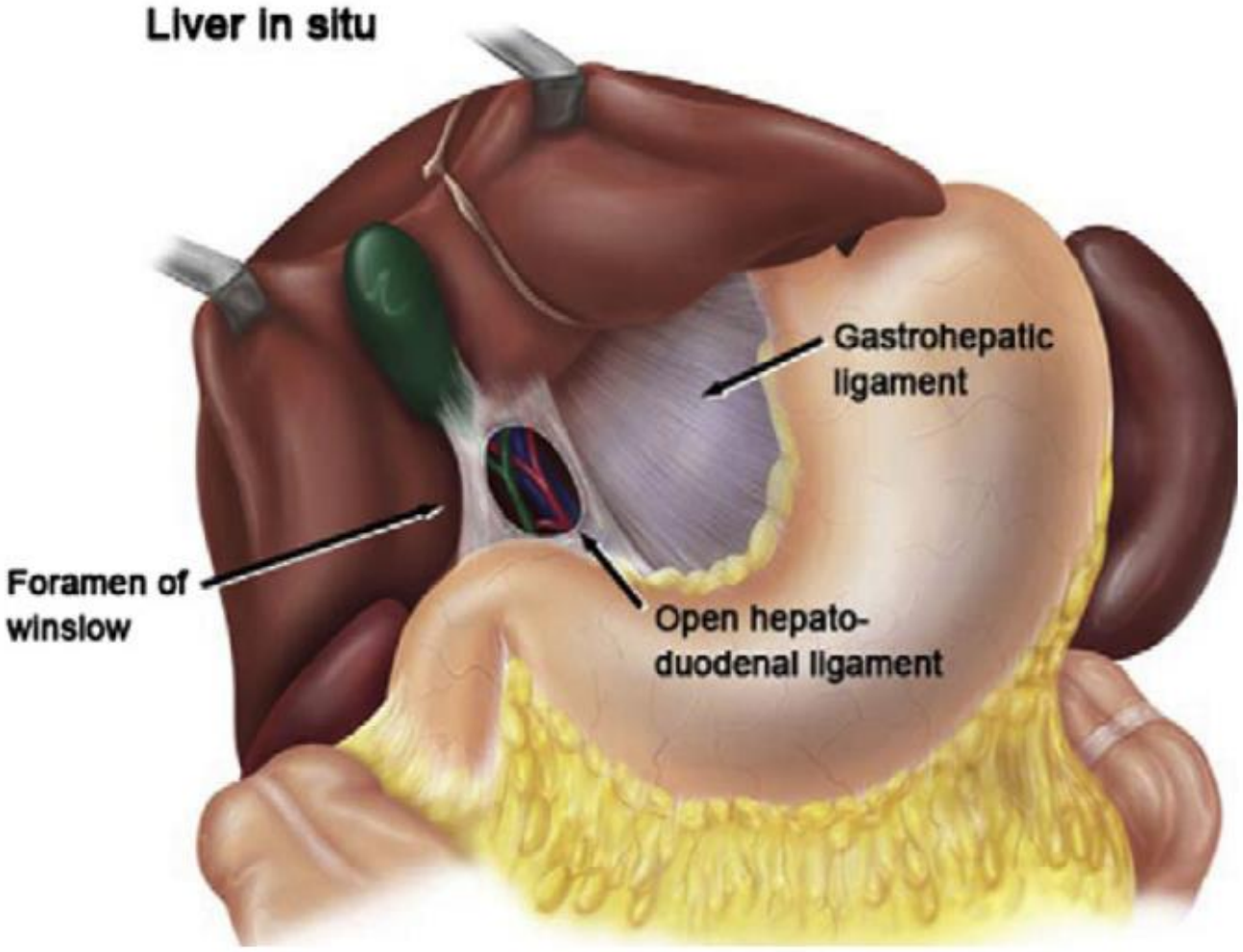


Figure 3. Association of stomach, porta hepatis, and hepatic flexure to the Liver. *From* Brunicardi FC, Andersen DK, Billiar TR, et al. Schwartz's principles of surgery. 9th edition. New York: McGraw-Hill Publishing; 2010. p. 31–3; with permission.

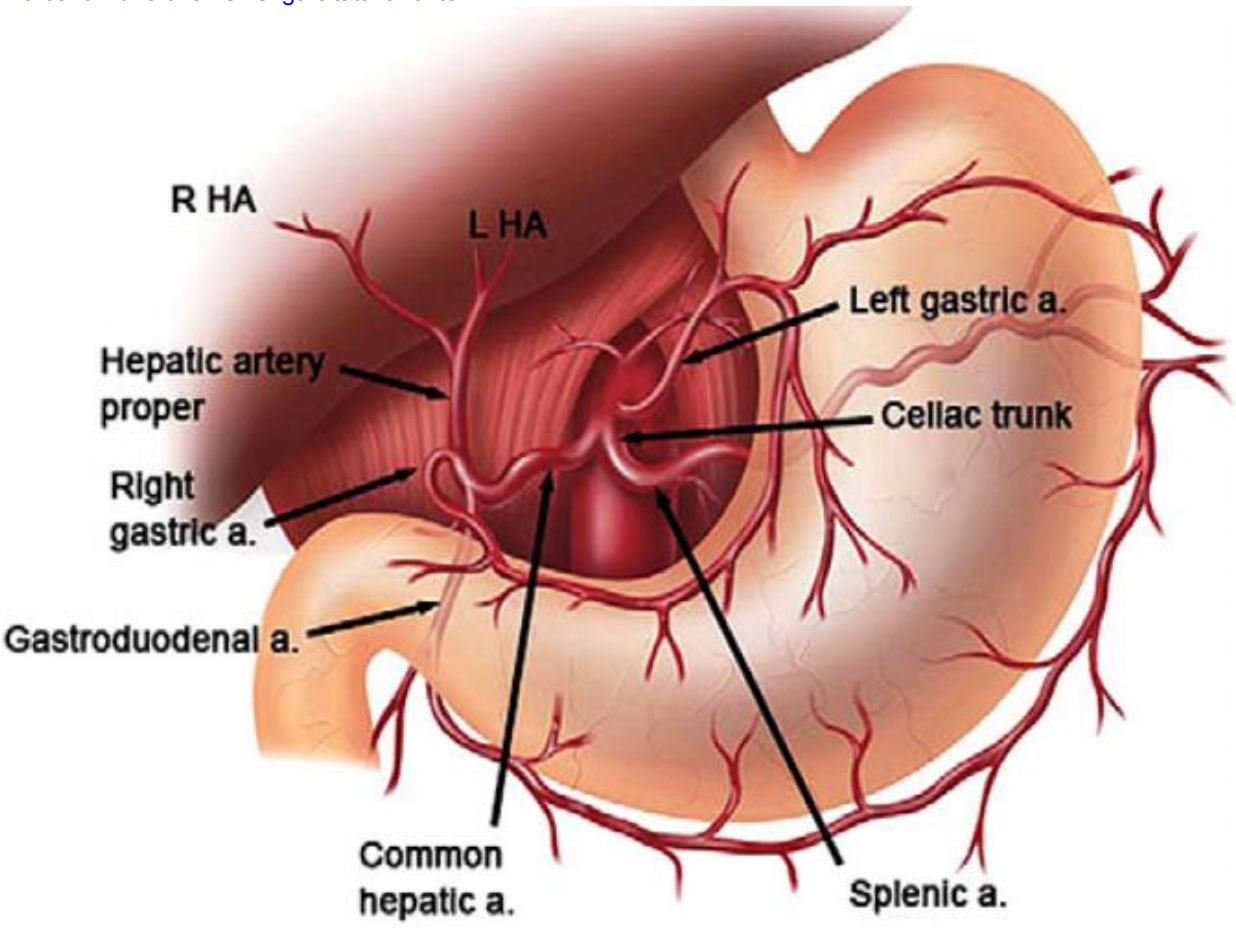


Figure 4. Common hepatic arterial configuration. HA, hepatic artery. *From* Brunicardi FC, Andersen DK, Billiar TR, et al. Schwartz's principles of surgery. 9th edition. New York: McGraw- Hill Publishing; 2010. p. 31–4; with permission.

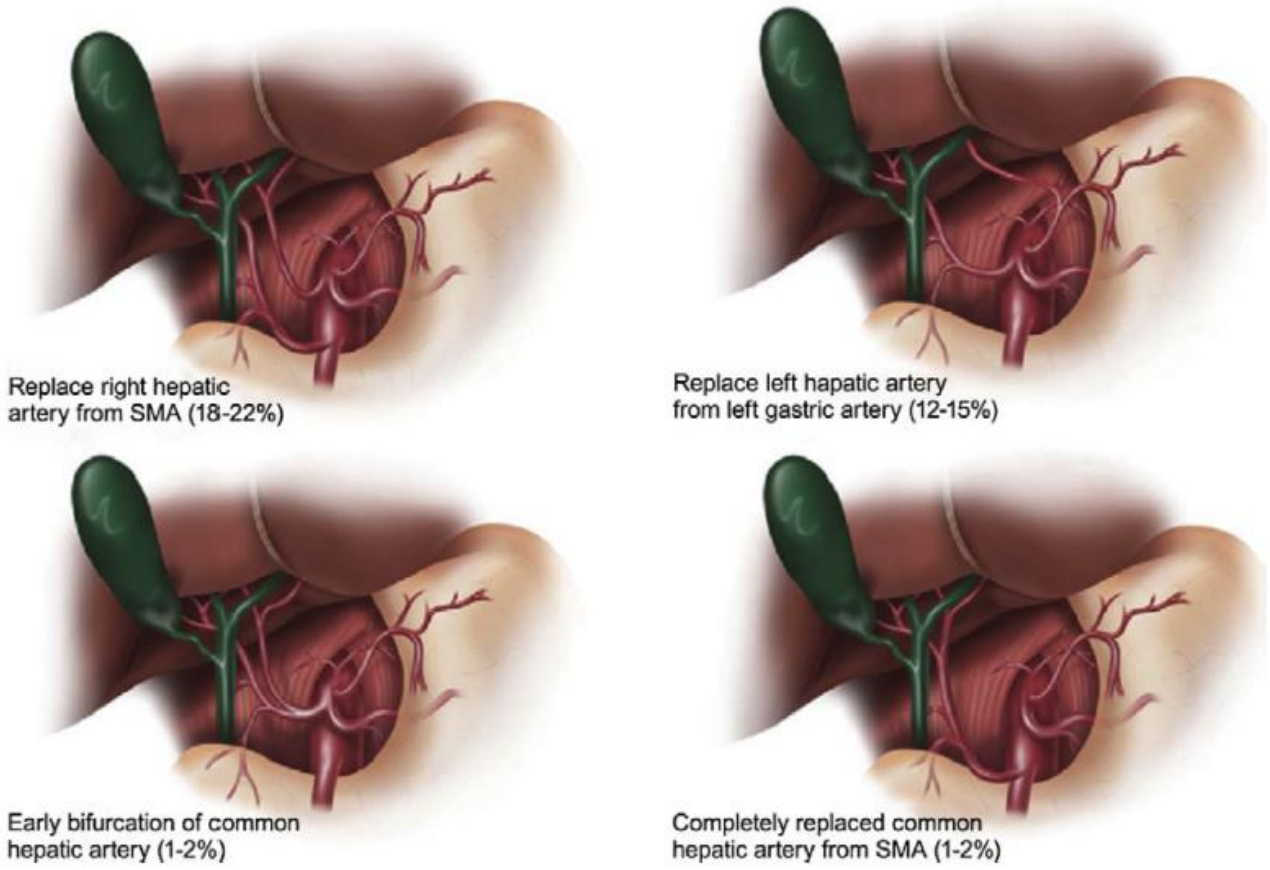


Figure 5. Common variations of hepatic vasculature. *From* Brunicardi FC, Andersen DK, Billiar TR, et al. Schwartz's principles of surgery. 9th edition. New York: McGraw-Hill Publishing. P. 31–4; 2010.

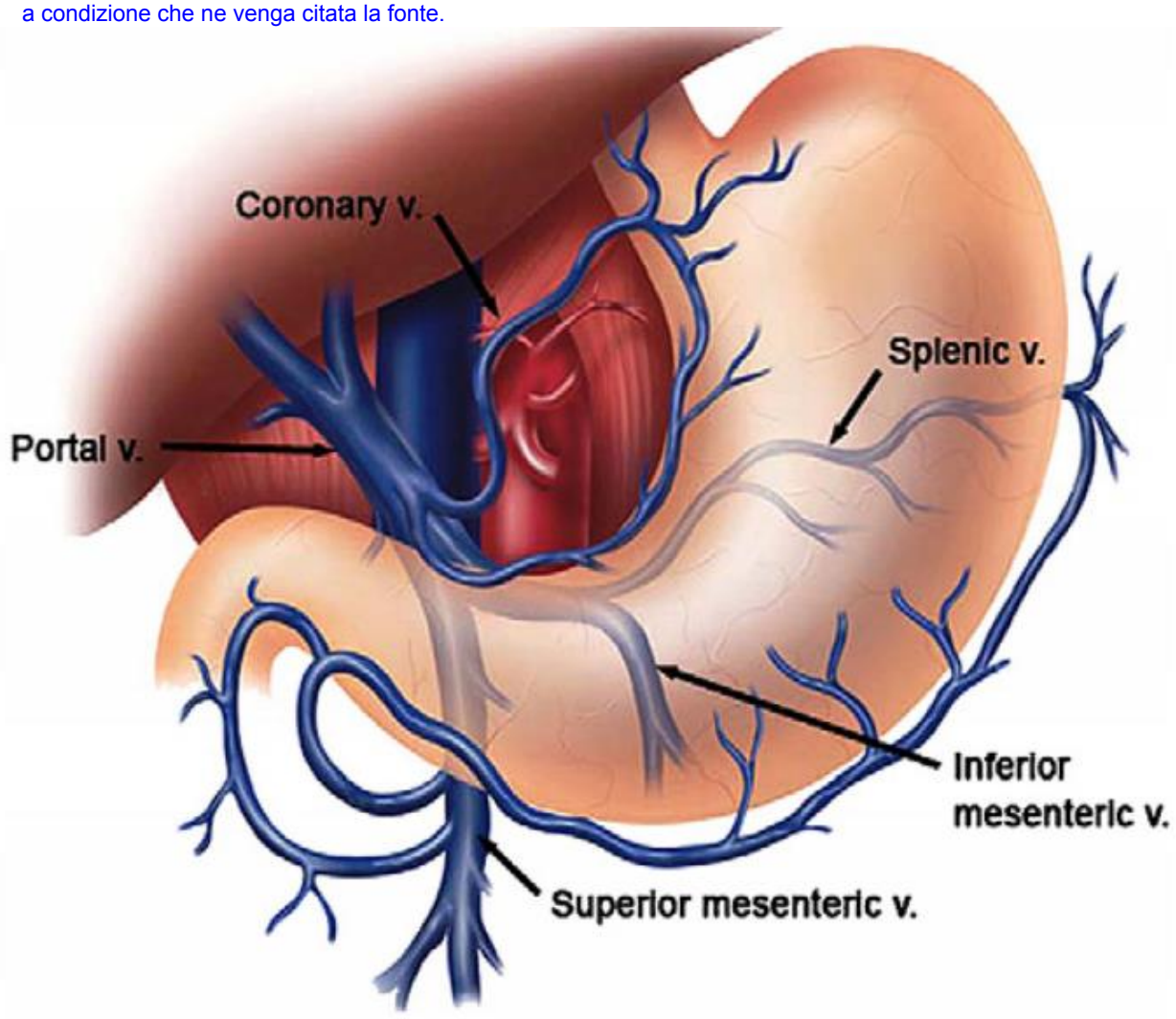


Figure 6. Portal vein and the hepatic venous vasculature inflow. *From* Brunicardi FC, Andersen DK, Billiar TR, et al. Schwartz's principles of surgery. 9th edition; McGraw-Hill Publishing. p. 31–5; 2010.

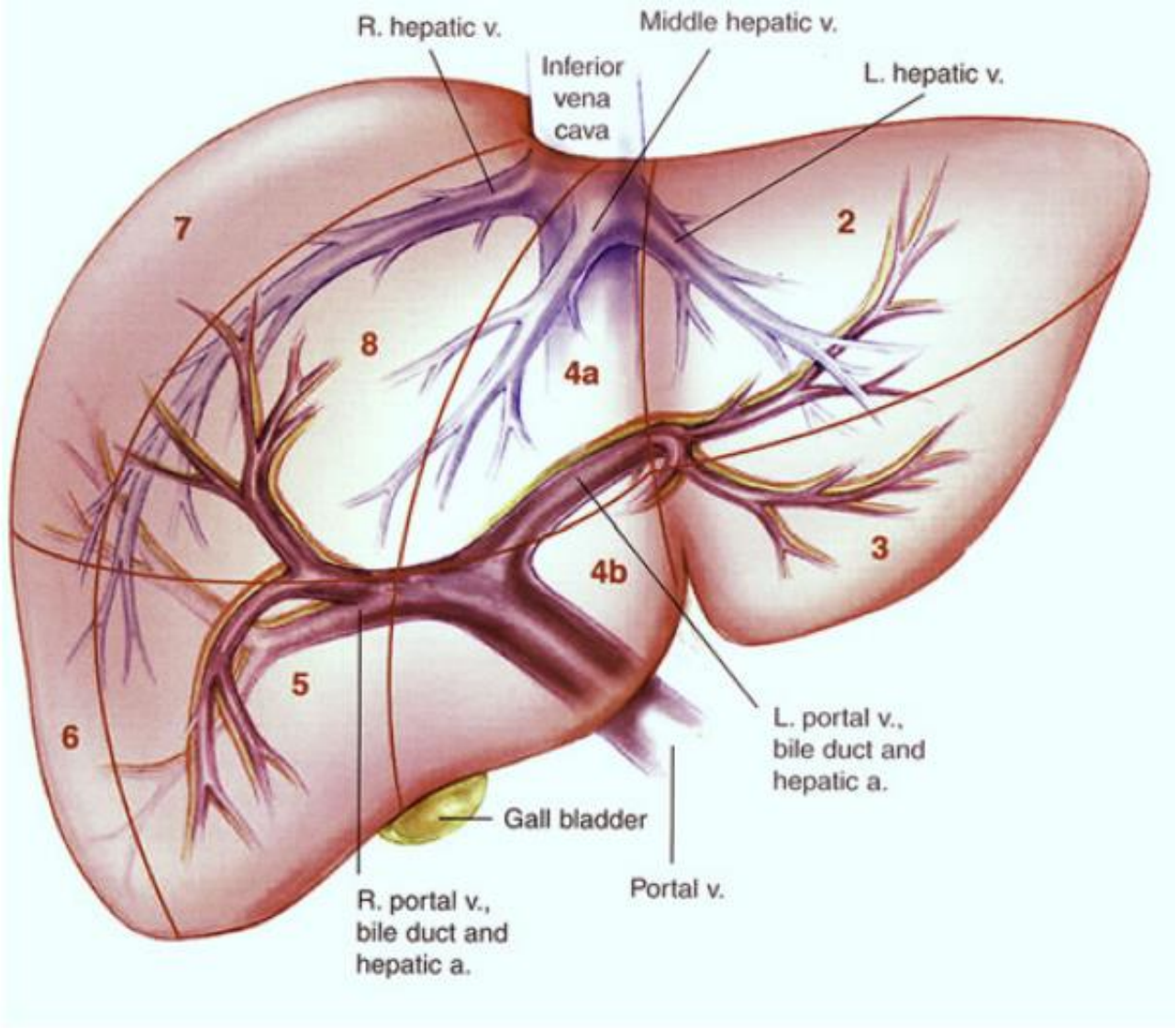


Figure 7. Intrahepatic vascular and biliary anatomy, anterior view. *Adapted from* Cameron JL, Sandone C. Atlas of gastrointestinal surgery, vol. 1. 2nd edition. Hamilton (ON): BC Decker; 2007. p. 121 [Fig. 1]; the People's Medical Publishing House—USA, Shelton, CT; with permission.

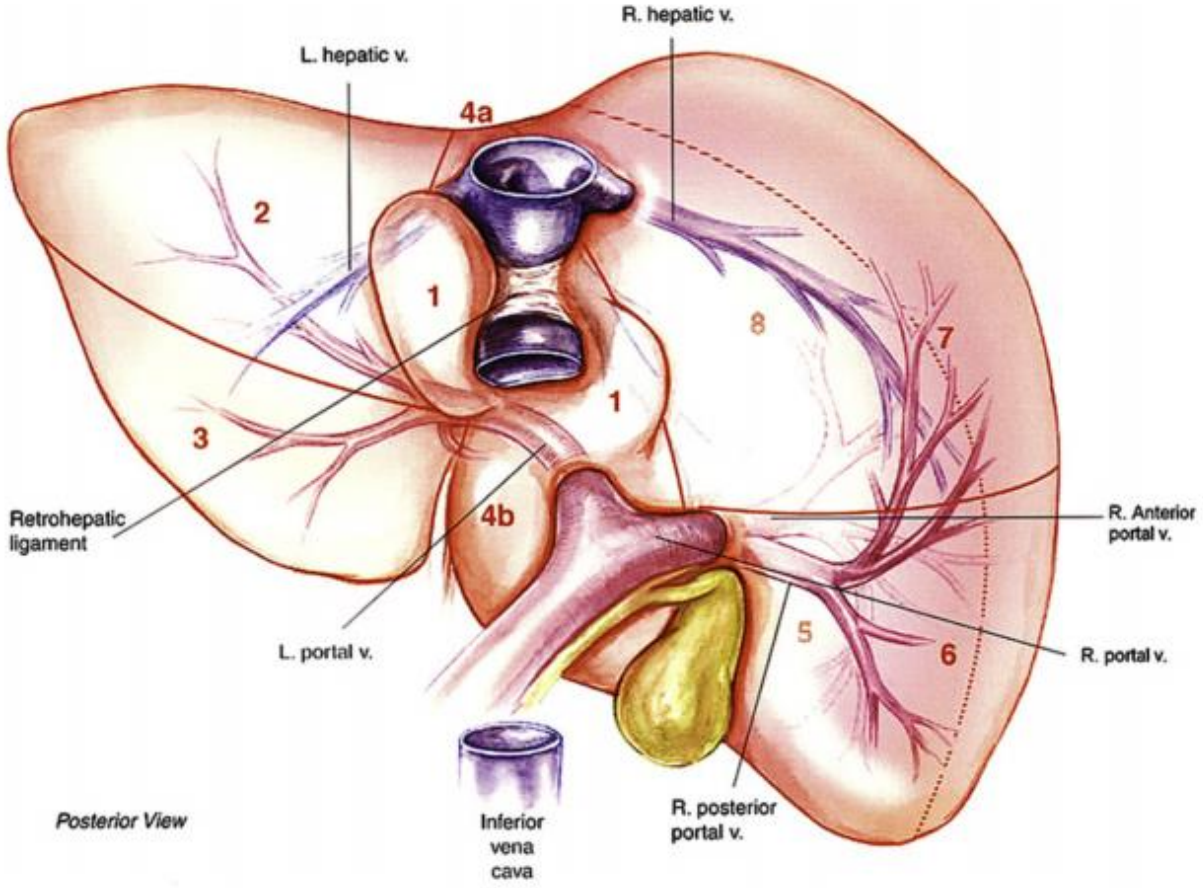


Fig. 8.

Intrahepatic vascular and biliary anatomy. posterior view. *Adapted from* Cameron JL, Sandone C. Atlas of gastrointestinal surgery, vol. 1. 2nd edition. Hamilton (ON): BC Decker; 2007. p. 124 [Fig. 2]; the People's Medical Publishing House—USA, Shelton, CT; with permission.

CHAPTER 3: HISTORY OF NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)

History of NAFLD Histopathology

- Before 1980

Addison was the first to describe fatty liver in 1836 [68]. Subsequently, for decades, pathologists pinpointed the similarities of liver histology changes seen in diabetic and morbidly obese individuals with those of alcoholics. In 1838, in autopsy specimens, the pathologist Rokitansky documented hepatic fat accumulation that might be causative of cirrhosis [69]. In 1884, Pepper described fatty infiltration of the liver in a diabetic patient [70]. In 1885, Bartholow reported a potential association between obesity and fatty liver [71]. In 1938, Connor described fatty liver infiltration that might led to the development of cirrhosis in diabetics. He reported on two cases of bleeding esophageal varices (one case was fatal owing to severe hemorrhage) in patients with diabetes and fatty liver. Perilobular fibrosis described in these patients was explained by both mechanical factors and tissue anoxia [72].

In 1958,Westwater and Feiner reported the histological findings of fatty infiltration of the liver in obese patients. In 1962, Thaler added a further clinical and pathological description of the disease. Since then, several reports in the 1950s–1970s pathologically documented the occurrence of fatty liver disease in obese and diabetic subjects [73].

- 1980 and Beyond

In 1980, the term nonalcoholic steatohepatitis (NASH) was coined by Ludwig et al., to describe the progressive form of fatty liver disease histologically resembling alcoholic steatohepatitis though observed in patients who denied any alcohol abuse. The majority of patients were obese women, and many were diabetic. The histopathological changes included lobular hepatitis, inflammatory infiltrates, Mallory bodies and focal necrosis with evidence of fibrosis in most specimens and cirrhosis in three patients 74. In 1983, Moran et al., extended these findings to obese children in whom steatohepatitis presented with abnormal liver enzymes and non-specific symptoms 75. Schaffner and Thaler were first to use the name "nonalcoholic fatty liver disease" in 1986 76. Over time, several histological scores for disease assessment have been developed and, currently, at least four main semi-quantitative scoring systems for the assessment of the histological features of NAFLD are available. The NAFLD activity score, comprised 14 histological features, 4 of which were evaluated semi-quantitatively: steatosis (0–3), lobular inflammation (0–2), hepatocellular ballooning (0–2), and fibrosis (0–4). Another nine features were recorded as present-or-absent. This score was developed by the NASH Clinical Research Network (NASH-CRN). The "Fatty Liver Inhibition of Progression (FLIP)" algorithm, which was developed by the FLIP consortium, is based on a scoring system (including steatosis, ballooning and lobular inflammation), the SAF score (steatosis, activity, fibrosis) 77. The so called "Brunt" system score included ten

histological variables to determine the inflammatory grading with a score for staging fibrosis 78. Finally, the pediatric NAFLD histological score was based on the evaluation of steatosis, ballooning, portal inflammation and lobular inflammation 79.

There is general consensus that a constellation of histological features is required for the histopathological identification of adult NASH, including steatosis, ballooning, lobular inflammation and perisinusoidal fibrosis. In contrast, there is no universal agreement among liver pathologists regarding the essential criteria for the diagnosis of NASH. In addition, compared to other histological features, such as fibrosis, the histological diagnosis of NASH exhibits a large inter- and intra-observer variability and sampling error, which is reflected by the widely ranging prevalence of NASH, from 1.4% to 20% of liver biopsies 80. This lack of reliability in the assessment of NASH may also a_ect NASH trials, by introducing patients who do not meet entry criteria, misclassifying fibrosis subgroups, and attenuating apparent treatment effects 81. For instance, in the sole Phase 3 clinical trial for NAFLD to date that showed significant results, obeticholic acid failed to demonstrate a significant impact on NASH resolution, though it had a significant effect on fibrosis 82-83. Future studies should identify reliable non-invasive tests for the prediction of NASH.

In this context, a panel of international experts from 22 countries across the globe recently proposed to abandon the simple and inaccurate dichotomous classification into 'NASH' versus 'non-NASH'. These authors, aiming at improving the assessment of severity of disease, argue that the gamut of liver lesions should rather be assessed as a

continuous and dynamic variable, such as is done in other diseases, therefore minimizing the negative implication of this conceptually wrong dichotomization.

Interestingly, steatosis may not persist during the progression of NAFLD, and rather may vanish in advanced cases of NAFLD-cirrhosis. This may lead to the blurring of the distinction between cryptogenic cirrhosis versus burned-out NAFLD-cirrhosis. Recently, various reports have demonstrated that features and the course of the two entities are different 84-86. Unfortunately, this group of patients is usually excluded from clinical trials, as they lack the key criterion of "presence of steatosis".

The international consensus panel clarified this aspect by proposing that patients with cirrhosis, even in the absence of typical histological features of steatohepatitis, should be considered as MAFLD-related cirrhosis if they meet at least one of the following criteria: past or present evidence of metabolic dysregulation (according to MAFLD criteria), with either documentation of MAFLD in previous biopsy or steatosis by imaging techniques.

History of Genetic NAFLD

NAFLD pathobiology has a high level of inheritability, and the genetic determinants of disease development and progression are increasingly recognized. Similar to other complex diseases, the genetic studies of NAFLD have passed through two major stages: the candidate gene approach first, followed by genome-wide association studies (GWAS) 87. The former approach is driven by hypotheses based on

the a priori knowledge of the biological functions regulated by candidate genes. Numerous variants of genes which can govern (therefore candidates) either susceptibility to or progression of NAFLD have been identified using this approach. However, most of these studies were underpowered owing to small size, which has been reflected by the inconsistency of published reports. The first GWAS in hepatology aimed at investigating the genetic basis of susceptibility to NAFLD dates back to 2008 88-89. Since then, hypothesis-free method-based discoveries, including GWAS, whole-genome and whole-exome sequencing have become the default methodology to determine genotype–phenotype associations. In these tests, correlations are performed between large numbers of single-nucleotide polymorphisms (SNPs), up to hundreds of thousands to over a million across the genome, and a single trait. This has led to an advancement in our understanding of the genetic underpinnings of NAFLD, with at least five variants in different genes having been robustly associated with the susceptibility to development and progression of NAFLD. These include: patatin-like phospholipase domain-containing protein 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), glucokinase regulator (GCKR), and hydroxysteroid 17_- dehydrogenase (HSD17B13) 89-93.

In addition, this approach helped in characterizing the genetic basis shared by NAFLD with other liver diseases as well as with other metabolic disorders, by identifying a role for variants in membrane bound O-acyltransferase domain-containing 7 (MBOAT7) 94-96, IFNL3/IFNL4 and FNDC5 in NAFLD. That said, it remains uncertain whether

"genetic NAFLD" is perfectly equivalent to "metabolic NAFLD" as far as, for example, cardiovascular risk is concerned 97.

In the post-GWAS era, we are currently harvesting the benefits of the GWAS discoveries, including the incorporation of genetics in diagnostic and prognostic models , with an emerging role for polygenic scores. In addition, genetic findings are well positioned to lead the path for modernization of the process of drug development, with recent evidence suggesting that a drug target with a genetic link has a double likelihood of success in clinical trials compared to other drugs that lack such a link 98-99. Finally, the era of phenome-wide association study (PheWAS), moving from investigating a single phenotype to considering multiple phenotypes, is emerging.

History of Clinical Correlates and Natural Course of NAFLD

- From the Metabolic Syndrome to NAFLD

The history of the metabolic syndrome is intriguing and complex. The first recognition of obesity and visceral adiposity as cardiovascular risk factors probably dates back to almost 2.400–260 years ago, Figure 1.

In 1765, the Italian medical genius, JB Morgagni, lucidly identified the principal features of what we would now define as metabolic syndrome. He reported on the anatomical basis of "android obesity" and associated such pathological findings with

hypertension, hyperuricemia, atherosclerosis and obstructive sleep apnea syndrome, long before the modern recognition of this syndrome.

This outstanding achievement descended from Morgagni's mechanistic view of human physiology and pathology. He envisaged health as the result of the well-balanced functioning of the various organs. Conversely, any disease resulted from specific tissue damage, and this is still largely accepted in contemporary medical sciences 100.

However, most contributions belong to the 20th century. During the 1920s, Austrian, Swedish and Spanish authors reported on the association of arterial hypertension, diabetes, obesity, hyperuricemia, and vascular disease. In the same decade, based on insurance data, it was observed that albuminuria/kidney disease, diabetes, cardio-circulatory disease, and high blood pressure clustered in overweight and obese individuals. In 1939, Himsworth identified two different types of diabetes and established an association between insulin resistance and risk of type 2 diabetes. In his seminal studies, conducted for almost 35 years, Vague and his group established a firm association between central distribution of body fat and unfavorable metabolic effects. However, it was not until the early 1980s that, owing to contributions by Kissebah and Bjorntorp, this concept became accepted 101.

The nomenclature of metabolic syndrome has been variable over time, including names such as hypertension–hyperglycaemia–hyperuricaemia syndrome, metabolic trisyndrome, plurimetabolic syndrome, syndrome of affluence, syndrome X, deadly quartet and insulin resistance syndrome.

Collectively, these studies were deemed to be consistent with the notion that NAFLD was "the hepatic manifestation of the Metabolic Syndrome", which agrees with the popular motto that "fatty people have fatty livers".

- From NAFLD to the Metabolic Syndrome

A more recent line of research, however, has shown that the association of NAFLD with metabolic syndrome is mutual and bi-directional. For example, in the early 2000s, it became clear that surrogate indices of hepatic dysfunction predicted incident T2D and metabolic syndrome. Bringing these epidemiological data further, it was possible to conduct theoretical as well as meta-analytic studies, showing that NAFLD was indeed a potential precursor of T2D and metabolic syndrome and that the stage of fibrosis was a strong determinant of such a risk 102-106.

NAFLD and Cardiovascular Risk

The liver was deemed to harbor life and soul in ancient Middle Eastern cultures, thus assuming a significance similar to that which the heart holds in our contemporary Western society. On this historical background, a strong link between NAFLD and cardio-metabolic risk has recently been identified 106-109.

In 1995, Lonardo et al. hypothesized that NAFLD could be a clue that is useful in detecting cardiovascular disease [11]. In 2004 and 2005, Targher et al. were first to report that NAFLD was significantly associated with early carotid atherosclerosis in healthy men, and an increased risk of cardiovascular disease in patients with T2D, independent of classical risk factors, and that the occurrence of metabolic syndrome could account for this, to a partial extent. Moreover, these authors also identified the stage of liver fibrosis as an independent predictor of carotid intima-media thickness, after the adjustment for potentially confounding factors such as metabolic syndrome. Since 2005, several studies have confirmed that NAFLD is strongly associated not only with subclinical atherosclerosis [134], but also with major cardiovascular events. In 2016, Targher et al., by meta-analyzing 16 unique, observational studies, enrolling a total of 34,043 adult individuals (36.3% had NAFLD), and evaluating nearly 2600 CVD events (>70% of which were CVD deaths) followed-up over a median period of 6.9 years, found that NAFLD patients, compared to controls without NAFLD, exhibited an increased risk of fatal and/or non-fatal CVD events. Moreover, those individuals who had "more severe" NAFLD, defined based on imaging techniques plus either elevated serum gamma glutamyltransferase concentrations or high NAFLD fibrosis score or high 2-deoxy-2-[fluorine-18] fluoro-D-glucose uptake on positron emission tomography, or by biopsy-proven fibrosis stages, were also more likely to develop fatal and non-fatal events of cardiovascular disease 110-114.

Therefore, modern studies seemingly confirm the historical notion that the liver is involved in cardiocirculatory physiopathology.

NAFLD and Cancer

By the early 2000s, it had already become clear that NAFLD was associated with both hepatic and extra-hepatic cancers.

- Hepatocellular Carcinoma

In 2002, two seminal studies reported on the risk of hepatocellular carcinoma (HCC) developing in the setting of NAFLD.

Bugianesi et al., by retrospectively identifying 44 patients with HCC occurring in the setting of cryptogenic cirrhosis (CC) out of 641 cirrhosis-associated HCCs, observed that hypertriglyceridemia, diabetes, and normal aminotransferases were the risk factors independently associated with HCC arising in CC, suggesting that HCC may represent a late complication of NASH-cirrhosis. Marrero et al., by studying 105 consecutive cases of HCC, reported that either histological or clinical features associated with NAFLD were common among patients with CC; moreover, HCCs manifesting among patients with CC were larger at diagnosis given that they were less likely to have undergone HCC surveillance, and therefore these were less likely to be candidates for surgical or local ablative therapies.

Presently, the development of HCC in a subset of individuals is a definite feature of the natural course of NAFLD. A meta-analytic review reported that, compared to other etiologies of liver disease, in non-cirrhotic subjects, those with NASH have a higher risk

of HCC. The risk factors for the development of HCC in those with NAFLD include genetics, lifestyle, liver-related and metabolic determinants 115-119.

- NAFLD and Extra-Hepatic Cancer

In their pioneer study, Sørensen et al., by using the Danish National Registry of Patients, compared the Danish general population data of 7326 individuals who had received a hospital diagnosis of: alcoholic (ICD-8 _ 571.10), nonalcoholic (ICD-8 _571.11), or unspecified fatty liver (ICD-8 _ 571.19) at least once during the 16-year study period. Data have shown that patients with nonalcoholic/unspecified fatty liver had an increased risk of pancreatic cancer (standardized incidence ratio (SIR) 3.0; 95% CI, 1.3–5.8; vs. SIR 1.5; 95% CI, 0.7–3.0) and kidney cancer (SIR 2.7; 95% CI, 1.1–5.6) 120.

Presently, a variety of extra-hepatic cancers, including colorectal adenoma and carcinoma, are increasingly identified as a systemic manifestation of NAFLD. Recent data suggest that NAFLD—more than obesity—is associated with an increased risk of extra-hepatic cancers, such as those of the gastrointestinal tract and uterus. A meta-analytic review of observational studies of asymptomatic individuals submitted to colonoscopy, owing to screening purposes reported that NAFLD was independently associated with a mildly increased risk of incident and prevalent colorectal adenomas and cancer. Various pathogenic mechanisms underlie the association of NAFLD with large bowel carcinogenesis, including sub-clinical systemic inflammation, IR, adipokines, bile acids and liver fibrosis 121-126.

History of Guidelines on NAFLD Issued by Scientific Societies

Over time, scientific societies from di_erent geographic areas have issued guidelines focusing on the criteria for diagnosis and management of NAFLD in adults, aimed at regulating clinical decision making. It is notable that a gap of decades separates the first clinico-pathological recognitions of NAFLD from recommendations issued by scientific societies. Probably, this mirrors the initial scarcity of evidence-based data to support strong recommendations. Distinctive features of the wide spectrum of NAFLD include expanding epidemiological trajectories, continuous progress in non-invasive diagnostic tools, as well as findings from basic research and clinical therapeutic trials of novel candidate drug regimens. All these concur in rendering publications and the updating of NAFLD guidelines a formidable multidisciplinary effort and an ongoing challenge for scientific hepatological societies. The first NAFLD guidelines were released by the Asian Pacific Association Study of the Liver (APASL) in 2007. This document was a summary of proposals by the Asian–Pacific Working Party for NAFLD, and was accompanied by reviews which summarized and annotated evidence and rationale supporting recommendations. It was an informative e_ort directed at clinicians regarding a new globally expanding disease. Interestingly, these authors were able to find some common grounds in NAFLD management, although strong evidence was lacking at that time. This first document proposed by Asian scientific societies paved the way for the publication of clinical practice guidelines for NAFLD in Europe.

In 2010, the European Association for the Study of the Liver (EASL) issued a position statement that summarized the proceedings of the 2009 EASL Special Conference on NAFLD/NASH. This seminal article proposed expert opinion regarding different aspects of the clinical care of NAFLD patients.

In 2012, a NAFLD guidelines document was published as a collaborative effort from the three major American hepatological societies: American Association for the Study of Liver Diseases (AASLD), American College of Gastroenterology and American Gastroenterological Association.

These comprehensive guidelines included an extensive scientific literature search and followed the standard Grading of Recommendation Assessment, Development and Evaluation (GRADE) methodology.

To complete this first set of international NAFLD guidelines, in 2014, TheWorld Gastroenterology Organization published a global NAFLD guidelines document, which is unique in following a resource-sensitive approach, i.e., a hierarchical set of diagnostic, therapeutic, and management options to deal with risk and disease, ranked by the resources available (Cascade). Between 2007 and 2014, either consensus statements or practice guidelines based on the recommendations of national societies were also issued. These include: the Italian Association for the Study of the Liver (AISF) in 2010, the Chinese Association of The Study of Liver Disease in 2011, the Korean Association for the Study of the Liver in 2013, and the Japanese Society of Gastroenterology and the Japanese Society of Hepatology in 2015 126-135.

The abundance and worldwide circulation of international and national guidelines witness that NAFLD is a global challenge. Concurrently, the high number and scientific standard of basic studies, clinical trials and informative review articles collectively attest that NAFLD remains an open and evolving paradigm for clinicians, needing further multidisciplinary approaches aimed at addressing the pathogenic heterogeneity, the multiple metabolic risk factors and the rapid epidemiological diffusion of disease. Major breakthroughs in our understanding of disease and evolving the medical practice fully justify a continuous updating of guidelines. Between 2016 and 2018, EASL, APASL and AASLD published the update of their first set of clinical recommendations. In particular, EASL worked in collaboration with the European Association for the Study of Diabetes and the European Association for the Study of Obesity, in developing the first multidisciplinary clinical practice guidelines on NAFLD in 2016. The 2016 EASL guidelines pay special attention to NAFLD screening in the population at risk. In 2018, APASL and AASLD published new consensus statements based on the most recent evidence 136-139.

Moreover, additional national societies either published novel or updated previous documents or guidelines. This is the case for NICE guidelines in 2016, AISF in 2017 and the Spanish Association for the Study of the Liver in 2018 140.

The comparative analysis of NAFLD guidelines is an informative academic practice, identifying both shared and diverging key points. The most updated of such comparative studies clearly highlights differences in the definition of alcohol threshold, choice of screening methods, identification of the best non-invasive tool for detecting liver

fibrosis and the discussion of different pharmacological approaches 141. There is general agreement regarding the notion that non-invasive tools such as NAFLD fibrosis score (NFS) and Fibrosis 4 score (FIB-4) and transient elastography or MRI should be used to detect patients with significant liver fibrosis. Moreover, scientific societies also agree that lifestyle changes, including healthy diet, habitual physical activity and weight loss are the mainstay of treatment. However, global management of NAFLD patients still varies across different geographical areas and different national healthcare systems. It is expected that translation into clinical practice of those shared recommendations may result in improving homogeneity in NAFLD management, as well as improved outcomes in clinical trials.

Although NAFLD has epidemic proportions in adults, children are not spared either 142. Additionally, pediatric NAFLD has distinctive histological and pathogenic features, and is an ever escalating cause of chronic liver disease, with the potential of impacting health outcomes in adolescents and young adults 143. This justifies the publication of NAFLD guidelines from pediatric scientific societies.

In 2017, practice guidelines on this topic were published by the North American Society for Pediatric Gastroenterology, Hepatology, Nutrition (NASPGHAN) and the update of AASLD guidelines on NAFLD included a pediatric section; this is a significant step towards providing diagnostic and therapeutic tools to optimize clinical care in children. The open questions in children are similar to those in adult populations: the identification of risk factors, screening strategies and screening tests, reference standard

for the diagnosis, non-invasive biomarkers and imaging; lifestyle modifications as the first-line approach 144.

History of General, Cellular and Molecular Pathogenesis of NAFLD and NASH

Our understanding of the level of complexity of NAFLD pathogenesis has increased over time. While the earliest view had indicated the mechanistic development of steatohepatitis as a simple "two-hit" phenomenon, i.e., cell insults such as oxidative stress, lipid oxidation and inflammation superimposed on steatosis caused by IR 145, subsequent theories have clearly elucidated a more sophisticated level of complexity. In their seminal paper, Tilg and Moschen proposed that, irrespective of whether inflammation chronologically precedes or follows steatosis, many parallel hits of intestinal and/or adipose tissue origin, endoplasmic reticulum stress, (adipo)cytokines and innate immunity act in concert to regulate the distinctive features of NASH 146. This "multiple hits hypothesis" continues to maintain its scientific credibility 147.

It would be difficult or even impossible to summarize here all the individual scientific contributions that, over time, have facilitated a more in-depth understanding of NAFLD and NASH pathogenesis.

CHAPTER 4: AIMS OF THE STUDY

- Identify in obese patients those risk factors determining the progression to SLD and helpful in targeting screening and follow-up strategies for obese patients.
- Identify which genetic polymorphisms can be adopted in combined predictive models to stratify risk of SLD in obese patients.

CHAPTER 5: MATERIALS AND METHODS

METHODS

Study population and data collection

We used data from the UK Biobank, a large prospective cohort including over 500,000 participants (age 40-69 years) recruited between 2006-2010 from 22 assessment centers throughout the UK. Study design and methods of the UK Biobank have been described in detail previously 148. Potential participants were identified from the National Health Service patient registers. At the baseline assessment visit, they completed a touch-screen self-administered questionnaire and a computer-assisted interview regarding medical history, current pharmacological therapy, sociodemographic characteristics, smoking status, alcohol consumption, dietary habits, physical activity and family history of major diseases. Baseline anthropometric measures (e.g. height, weight and waist circumference) were assessed by trained staff using standardized procedures. Blood samples were collected for genome-wide

genotyping and biochemical analyses, including serum glucose, total cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and albumin (AU5800, Beckman Coulter). The protocol for samples collecting, processing and storage was developed using a highly automated and validated approach 149. Further information about the study protocol and methods is available in the UK Biobank website (https://www.ukbiobank.ac.uk/). The UK Biobank study has been approved by the North West Multicenter Research Ethics Committee (reference number 11/NW/0382). All participants provided written informed consent to the study.

Sample selection

From the total study population (N 502,536), we excluded subjects with: 1) self-reported history of liver disease, alcohol abuse or excessive alcohol consumption (≥30 g/die and ≥20 g/die for men and women, respectively); 2) hospital diagnosis of liver disease occurred before the baseline visit and defined according to the International Classification of Diseases 10th edition (ICD-10); 3) diagnosis of any cancer (except for precancerous conditions of the cervix) both self-reported or based on cancer registry and occurred before the baseline assessment visit. Thereafter, we removed participants with non-European descent and those with withdrawn consent (N=30). Finally, subjects with missing BMI data were excluded. A total of 330,046 participants were included for the final analyses [Obese 80,224 (24.3%), Overweight 138,125 (41.9%), Normal

111,697 (33.8%)]. Details of baseline exclusion criteria have been provided in Tables S1-S2.

Baseline covariates and comorbidities

Height and weight were measured using the Seca 202 height measure (Seca, Hamburg, Germany) and the Tanita BC-418 MA body composition analyser (Tanita Europe, Amsterdam, Netherlands), respectively. Body mass index (BMI) was calculated by dividing the weight (kg) by the square of the height (m^2). Waist circumference was measured at the umbilicus level using the Wessex non-stretchable sprung tape measure (Wessex, UK). Smoking status was categorized into two groups: current smoking and never/former smoking. Baseline type 2 diabetes was defined by at least one of the following criteria: 1) self-reported history of type 2 or unspecified diabetes; 2) hospital diagnosis of type 2 or unspecified diabetes occurred before the baseline assessment visit (ICD-10 E11, E14); 3) current insulin treatment and/or use of oral hypoglycemic drugs; 4) serum glucose level ≥ 11.1 mmol/L (200 mg/dL); 5) HbA1c ≥ 48 mmol/mol (6.5%). Baseline dyslipidemia was defined as self-reported history of high cholesterol or use of lipid-lowering drugs. Similarly, baseline hypertension was defined as self-reported history of hypertension or use of anti-hypertensive drugs. Baseline cardiovascular disease was defined as self-reported history of angina, myocardial infarction, stroke or transient ischemic attack.

Genotyping

Detailed information about genotyping and arrays used in the UK Biobank study has been provided elsewhere 150. PNPLA3 rs738409 C>G (p.I148M), TM6SF2 rs58542926 C>T (p.E167K), MBOAT7 rs641738 C>T, GCKR rs1260326 C>T (p.P446L) and HSD17B13 rs72613567:TA were genotyped using two very similar arrays (i.e., Affymetrix UK BiLEVE and UK Biobank Axiom arrays) and coded as 0, 1 or 2 for non-carriers, heterozygous carriers and homozygous carriers of the minor allele, respectively. Finally, to summarize the impact of genetic predisposition to fatty liver, we exploited the PRS-HFC, which we recently developed to predict the inherited predisposition to hepatic fat accumulation quantified by the gold standard H1-MRS in the general population 151. The PRS-HFC is calculated by summing the number of the risk alleles in PNPLA3, TM6SF2, MBOAT7 and GCKR weighted by their effect size, using the formula: 0.266*PNPLA3 + 0.274*TM6SF2 + 0.065*GCKR + 0.063*MBOAT7 variant alleles.

Outcome ascertainment

Follow-up data on health-related events and mortality were obtained through linkage of the National Health Service records, including in-hospital admissions, death register and cancer register (UK Biobank data-fields 41270, 40001, 40002, and 40006). The outcome of interest was incident SLD, defined as a composite diagnosis of

cirrhosis, decompensated liver disease (i.e., esophageal varices with or without bleeding, portal hypertension, hepatorenal syndrome, liver failure), hepatocellular carcinoma and/or liver transplantation (ICD-10 C22.0, I85.0, I85.9, K70.3, K70.4, K72.1, K72.9, K74.1, K74.2, K74.6, K76.6, K76.7, Z94.4) in any of the aforementioned records. A list of all the diagnoses used to define SLD is presented in Table S3. The follow-up began at the date of baseline assessment visit and ended at the date of SLD, death, competing non-NAFLD diagnoses or last update of the registries (31 January 2018), whichever occurred first. Competing non-NAFLD diagnoses were considered as the occurrence of hospital diagnosis of chronic viral hepatitis, Wilson disease, hemochromatosis, drug-induced liver injury, autoimmune hepatitis, inflammatory liver diseases and/or chronic biliary disorders (ICD-10 B18, B19, E83.0, E83.1, K71, K74.3, K74.4, K74.5, K75.2, K75.3, K75.4, K75.8, K75.9) during the follow-up.

Statistical analysis

For descriptive statistics, categorical variables were shown as number and proportion, while continuous variables were shown as mean with standard deviation (SD) or median with interquartile range (IQR), as appropriate. Cox proportional hazard regression models were fitted to investigate the impact of obesity, and factors associated with the occurrence of SLD in obese subjects. The strength of associations was expressed by means of hazard ratio (HR) with 95% confidence intervals (CI). Multivariable models were carried out to correct for potential confounders, and

including variables associated with outcome based on prior knowledge, or with a p value <0.1 at univariate analysis. The proportional hazard assumption was verified through the inspection of the Schoenfeld residuals. Analyses were stratified according to gender. The predictive performances of different WC classes for the development of SLD were then estimated calculating general accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for incident SLD. All analyses were conducted with R statistics 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Table S1. Definition of self-reported history of liver disease (data-field 20002)

Code	Description
1136	liver/biliary/pancreas problem
1141	oesophageal varices
1155	Hepatitis
1156	infective/viral hepatitis
1157	non-infective hepatitis
1158	liver failure/cirrhosis
1159	bile duct disease
1408	alcohol dependency
1506	primary biliary cirrhosis
1507	Haemochromatosis
1578	hepatitis a
1579	hepatitis b
1580	hepatitis c
1581	hepatitis d
1582	hepatitis e
1604	alcoholic liver disease / alcoholic cirrhosis

Table S2. ICD-10 codes used to define baseline liver disease

Diagnosis	ICD-10
Viral hepatitis, chronic	B18
Viral hepatitis, unspecified	B19
Hepatocellular carcinoma	C22.0
Disorders of copper metabolism	E83.0
Disorders of iron metabolism	E83.1
Alcoholic liver disease	K70
Toxic liver disease	K71
Esophageal varices, bleeding	I85.0
Esophageal varices, not bleeding	I85.9
Liver failure, chronic	K72.1
Liver failure, unspecified	K72.9
Chronic hepatitis, not elsewhere classified	K73
Liver fibrosis and cirrhosis	K74
Portal hypertension	K76.6
Hepatorenal syndrome	K76.7
Ascites	R18
Nonspecific reactive hepatitis	K75.2
Granulomatous hepatitis, not elsewhere classified	K75.3
Autoimmune hepatitis	K75.4
Other specified inflammatory liver diseases	K75.8

Inflammatory liver disease, unspecified	K75.9
Fatty liver, not elsewhere classified	K76.0
Other specified diseases of liver	K76.8
Liver disease, unspecified	K76.9
Liver transplant status	Z94.4

Table S3. Diagnoses and ICD-10 codes used to define severe liver disease endpoint

Diagnosis	**ICD-10**
Hepatocellular carcinoma	C22.0
Esophageal varices, bleeding	I85.0
Esophageal varices, not bleeding	I85.9
Alcoholic liver cirrhosis	K70.3
Alcoholic liver failure	K70.4
Liver failure, chronic	K72.1
Liver failure, unspecified	K72.9
Hepatic sclerosis	K74.1
Hepatic fibrosis with hepatic sclerosis	K74.2
Liver cirrhosis, other and unspecified	K74.6
Portal hypertension	K76.6
Hepatorenal syndrome	K76.7
Liver transplant status	Z94.4

CHAPTER 6: RESULTS

After adoption of exclusion criteria, 80.224 persons with obesity (defined as BMI ≥ 30 kg/m²) were matched to 330.046 controls (normal weight or overweight). At baseline, mean age in obese persons was 56.9 years (standard deviation, 7.9), 55% were female, and the mean BMI was 34.1 kg/m² (standard deviation, 3.9), waist circumference mean was 104.9, smokers were 9%, diabetes mellitus was present in 12%, hypertension in 46% and dyslipidaemia in 31%.

In the obese group 318 persons with obesity (0.4%) developed severe liver disease, as compared with 379 of non-obese subjects (0.15%) p=0.019

Baseline characteristics according to the presence of obesity are presented in Table 1.

The cumulative incidence curves for severe liver disease in the 3 groups (normal weight, overweight and obese) in the overall and stratified by sex are presented in **Figure 1**, this means that there's a strong association between BMI and incidence of SLD.

Comparing the obese group vs non obese the risk for severe liver disease was increased in obese persons compared with controls (hazard ratio HR, 2.63 ; 95% confidence interval CI, 2.27-3.05), p<.001 in the unadjusted model.

In the model 1 adjusted for age, sex, diabetes mellitus, hypertension and dyslipidaemia remains a strong association between obesity and risk for severe liver disease (hazard ratio HR, 1.86 ; 95% confidence interval CI, 1.58-2.16).

A different pattern was observed in model 2 which includes WC (adjusted for age, sex, diabetes mellitus, hypertension, dyslipidaemia and waist circumference) (hazard ratio HR, 1.10; 95% confidence interval CI, 0.89-1.37) and model 3 adjusted only for WC (hazard ratio HR, 0.99; 95% confidence interval CI, 0.81-1.21) (**Table 2**), this means that with the same WC an obese persons has the same risk of non obese person to develop severe liver disease.

Considering only the obese population at follow up the subgroups of persons that developed SLD, mean age was 60 years (standard deviation ±6.7), 37% were female, BMI mean was 35.2 kg/ m² (standard deviation ±4.8), waist circumference mean was 112.1 cm (standard deviation ±11.1) and other characteristics of the obese population according to the incidence of severe liver disease at follow up are presented in **Table 3**. **Table 4** report univariate and multivariate logistic regression analyses in which are analyzed the risk factors associated with SLD in the overall population and **Table 5** stratified by sex. Among genetic factors, this group was enriched in carriers of the PNPLA3 rs738409 and TM6SF2 rs58542926 variants.

The risk factors associated with SLD across uni and multivariate models are represented by: age (hazard ratio HR, 1.05; 95% confidence interval CI, 1.03-1.07), p<.001; male sex (hazard ratio HR, 1.21; 95% confidence interval CI, 0.89-1.65), p=NS; BMI (hazard ratio HR, 0.98; 95% confidence interval CI, 0.94-1.02), p=NS; waist circumference (hazard ratio HR, 1.04; 95% confidence interval CI, 1.02-1.06), p<.001; diabetes mellitus (hazard ratio HR, 2.18; 95% confidence interval CI, 1.55-3.05), p<.001;

hypertension (hazard ratio HR, 1.34; 95% confidence interval CI, 1.03-1.75), p=0.03; smoke status (hazard ratio HR, 1.10; 95% confidence interval CI, 1.17-2.31), p<.001; glycated (hazard ratio HR, 1.09; 95% confidence interval CI, 0.96-1.23), p=NS; pnpla3 (hazard ratio HR, 1.59; 95% confidence interval CI, 1.33-1.9), p<.001; pnpla3r (hazard ratio HR, 2.62; 95% confidence interval CI, 1.82-3.79), p<.001; tm6sf2 (hazard ratio HR, 1.37; 95% confidence interval CI, 1.04-1.81), p 0.027; gckrr (hazard ratio HR, 1.45; 95% confidence interval CI, 1.09-1.93), p 0.01.

The waist circumference and diabetes are the variables which remain in both sexes across uni and multivariate models.

New WC cut-off have been recently proposed in the Consensus Statement from the IAS and ICCR working group on visceral obesity 152, and shows four thresolds for waist circumference stratified by BMI for white individuals (individuals with measurements higher than these values have an increased risk of future coronary events (based on 10-year risk of coronary events or the presence of diabetes mellitus). Waist circumference threshold indicating increased health risk within each BMI Category 152.

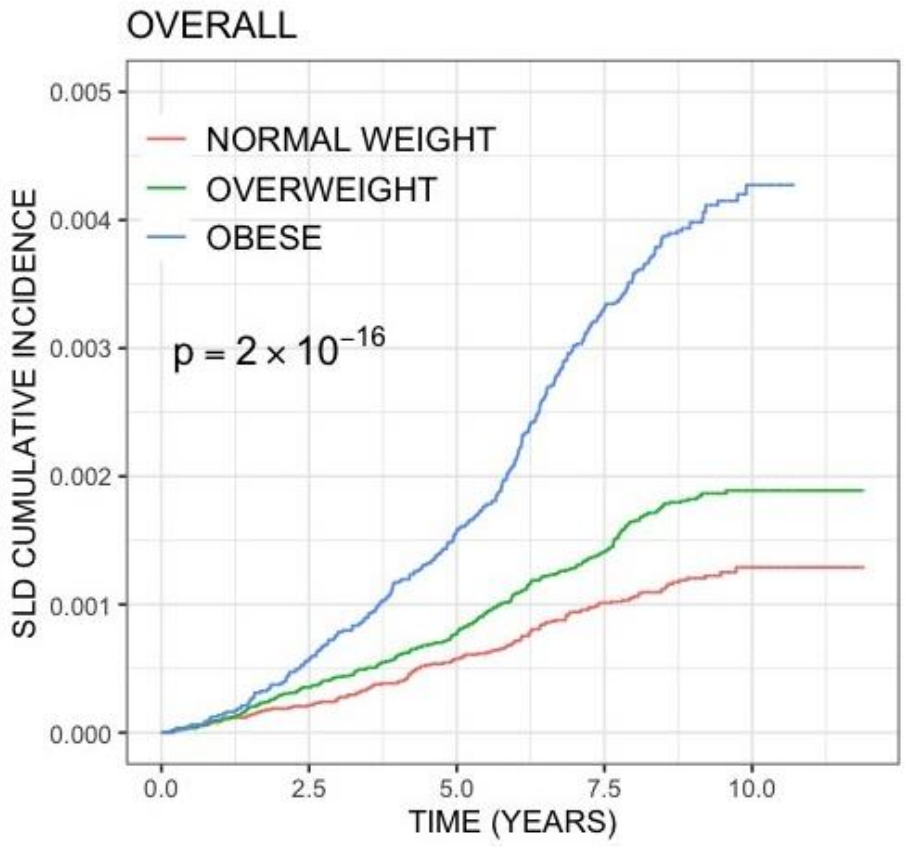
The SLD cumulative incidence in the overall population and stratified by sex showed that in both sexes, the use of BMI category-specific waist circumference thresholds improved significantly identification of obese at a high risk of future severe liver disease (**Figure 2**).

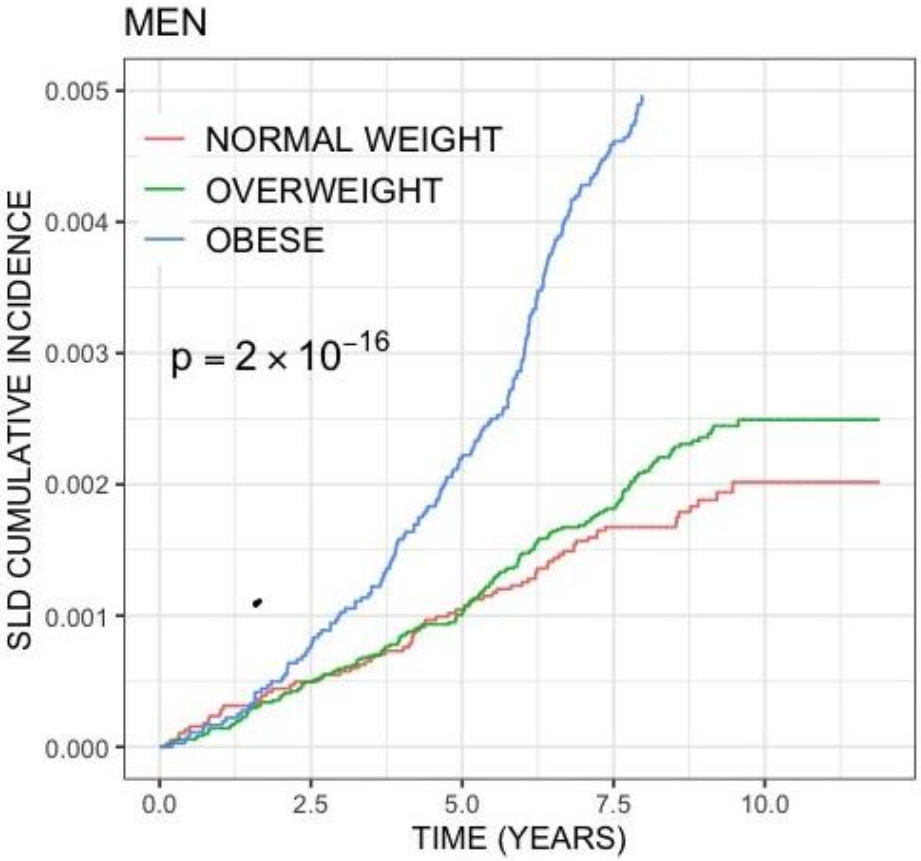
Subjects with obesity were divided according to three WC cut off values: 1) ≥ 88/102 (cm, male/female), 2) ≥ 105/110 (cm, male/female) and 3) ≥ 115/125 (cm, male/female) and they were further divided by class I obesity and class II and III (**Figure 2**).

If class II and III obesity still gives an increased cumulative risk compared to class I obesity in the first WC cut off value class (5.3 *vs* 3.7), this effect is lost at the higher WC cut off values 2 and 3. In fact, at WC cut off value class 2 no difference were observed in the SDL cumulative risk between class I or II-III obesity (6.6 *vs* 6.6) and more importantly, in subjects with the highest WC cut off value 3, those with class I obesity have a higher SDL cumulative risk compared with those with class II-III obesity (15.5 *vs* 8.6). The same direction is observed when stratifying by gender and it is more evident in males **(Figure 2)**. A WC/BMI ratio has been produced and, using tertiles of this parameter, a cumulative incidence of developing SDL has been calculated stratifying by the three obesity classes **(Figure 3).** Subjects in the highest tertile of WC/BMI ratio showed a higher cumulative incidence of SDL even at lower BMI classes. Subjects in the lowest tertile of the WC/BMI ratio showed a nearly absent cumulative incidence of SDL throughout the years (10 years) in the obesity class I and II, but this effect disappeared after 5 years in the in class III obesity **(Figure 3)**.

	All	Not Obese	Obese	
N	330046	249822 (75.69%)	80224 (24.31%)	-
Age (years), mean(SD)	56.5 (8.1)	56.4 (8.2)	56.9 (7.9)	1.8*10^-58
Female, n(%)	185171 (56%)	140965 (56%)	44206 (55%)	1.8*10^-51
BMI (Kg/m^2), mean(SD)	27.4 (4.9)	25.3 (2.7)	34.1 (3.9)	2.7*10^-7
Waist circumference	89.9 (13.6)	85.1 (10.3)	104.9 (11.2)	<10^-300
Smokers, n(%)	29085 (9%)	22013 (9%)	7072 (9%)	7.3*10^-1
Diabetes mellitus, n(%)	16476 (5%)	7121 (3%)	9355 (12%)	<10^-300
Hypertension, n(%)	94480 (29%)	57431 (23%)	37049 (46%)	<10^-300
Dyslipidaemia, n(%)	70626 (21%)	45436 (18%)	25190 (31%)	<10^-300
Incidence of SLD, n(%)	697 (0.211%)	379 (0.152%)	318 (0.396%)	1.9*10^-33

Table 1. Baseline characteristics according to the presence of obesity





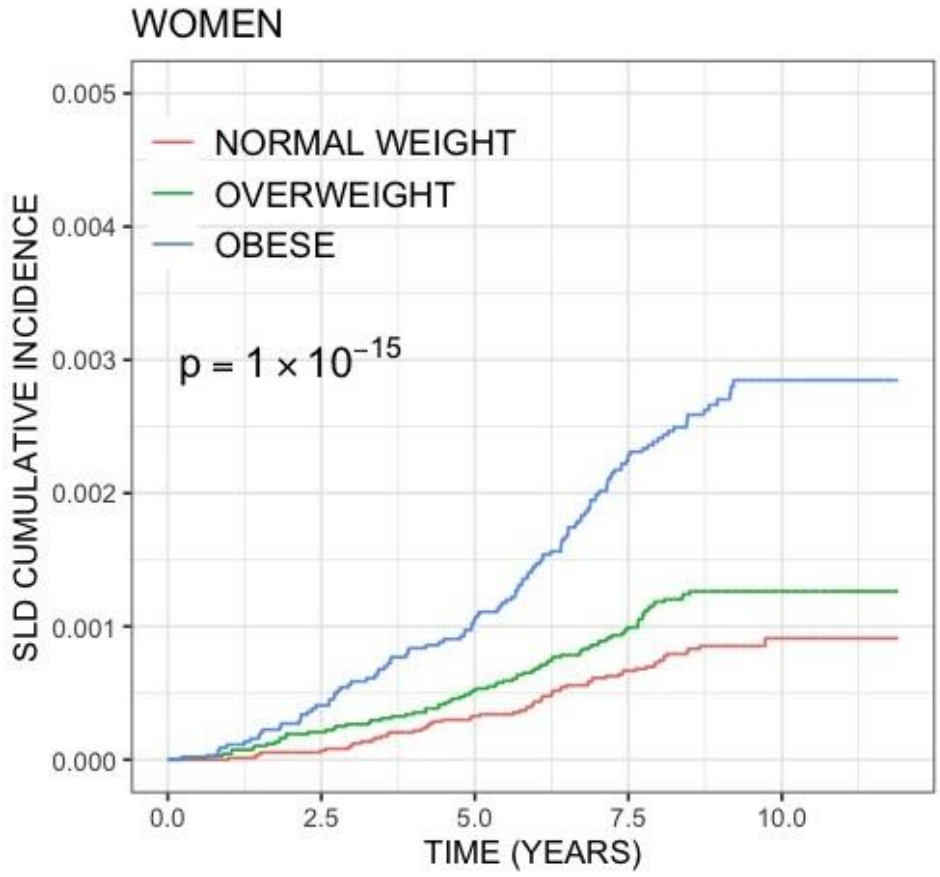


Figure 1. Cumulative incidence of SLD across groups of normal weight, overweight and obese individuals, in the overall population and stratified by sex. P values are log-rank p for trend.

	Risk for Severe Liver Disease		
	Overall	Men	Women
	HR(95%CI), p	HR(95%CI), p	HR(95%CI), p
Being Obese vs Not Obese			
Unadjusted model	2.63 (2.27-3.05), 5.1*10^-37	2.60 (2.15-3.13), 3.6*10^-23	2.61 (2.06-3.35), 7.8*10^-15
Adjusted model 1	1.86 (1.58-2.16) 4.0*10^-14	1.82 (1.49-2.23), 4.3*10^-9	1.89 (1.46-2.45), 1.8*10^-6
Adjusted model 2	1.10 (0.89-1.37) 3.9*10^-1	1.25 (0.95-1.64) 1.1*10^-1	0.89 (0.62-1.28) 5.3*10^-1
Adjusted model 3	0.99 (0.81-1.21), 9.2*10^-1	1.29 (0.987-1.69), 6.2*10^-2	0.88 (0.61-1.26), 4.8*10^-1

Note: Severe liver disease is defined as a composite endpoint that includes cirrhosis, hepatocellular carcinoma, liver failure, or death from any of these.

Model 1 adjusted for age, sex, diab, ipert, dislip

Model 2 adjusted for model 1 + waist circumference

Model 3 adjusted for only waist circumference

Table 2. Risk for severe liver disease across obese vs not obese

		Incident Severe Liver Disease		
	All	**SLD-**	**SLD+**	**P**
N	80224	79906 (99.6%)	318 (0.4%)	-
Age (years), mean(SD)	56.9 (7.9)	56.9 (7.9)	60 (6.7)	2.7*10^-12
Female, n(%)	44206 (55%)	44088 (55%)	118 (37%)	2.6*10^-2
BMI (Kg/m^2), mean(SD)	34.1 (3.9)	34 (3.9)	35.2 (4.8)	2.7*10^-12
Waist circumference (cm), mean(SD)	104.9 (11.2)	104.8 (11.2)	112.1 (11.1)	4.8*10^-23
Smokers, n(%)	7072 (9%)	7030 (9%)	42 (13%)	2.6*10^-3
Diabetes mellitus, n(%)	9355 (12%)	9239 (12%)	116 (36%)	2.0*10^-26
Hypertension, n(%)	37049 (46%)	36835 (46%)	214 (67%)	2.5*10^-7
Dyslipidaemia, n(%)	25190 (31%)	25012 (31%)	178 (56%)	4.8*10^-10
Cardiovascular disease, n(%)	7578 (9%)	7500 (9%)	78 (25%)	2.0*10^-8
HbA1c (%), median(IQR)	5.5 (5.3-5.8)	5.5 (5.3-5.8)	5.9 (5.4-6.6)	1.7*10^-17
ALT (U/L), median(IQR)	24 (18.1-32.9)	24 (18-32.9)	31.3 (23-49.6)	1.9*10^-31
AST (U/L), median(IQR)	25 (21.3-30.1)	25 (21.3-30)	33.4 (25.7-49.8)	1.1*10^-74
GGT (U/L), median(IQR)	31.9 (22.6-47.9)	31.8 (22.6-47.7)	70.7 (41.8-159.3)	5.5*10^-86
Alkaline phosphatase (U/L), median(IQR)	85.7 (72.3-101.5)	85.7 (72.3-101.4)	97.6 (77.2-120.7)	3.5*10^-26

Total bilirubin (mg/dL), median(IQR)	0.4 (0.4-0.6)	0.4 (0.4-0.6)	0.5 (0.4-0.7)	4.4*10^-5
Total cholesterol (mg/dL), mean(SD)	217.1 (47)	217.2 (47)	188.9 (50.3)	9.7*10^-16
LDL (mg/dL), mean(SD)	138.2 (35.5)	138.3 (35.5)	117.5 (36.6)	4.5*10^-15
HDL (mg/dL), mean(SD)	49 (11.7)	49 (11.7)	44.1 (11.1)	1.3*10^-6
Triglycerides (mg/dL), median(IQR)	167.9 (121.9-232.8)	167.9 (121.9-232.8)	171 (116.9-227.1)	2.2*10^-1
Albumin (mg/dL), mean(SD)	4.5 (0.3)	4.5 (0.3)	4.4 (0.3)	1.9*10^-11
Platelets (*10^9 cells/L), mean(SD)	258.1 (61.2)	258.2 (61.1)	223.5 (77.7)	5.9*10^-17
PNPLA3 rs738409 C>G (CC/CG/GG), n(%)	48113 (62%) / 26100 (34%) / 3592 (5%)	47959 (62%) / 25987 (34%) / 3556 (5%)	154 (51%) / 113 (37%) / 36 (12%)	2.6*10^-8
TM6SF2 rs58542926 C>T (CC/CT/TT), n(%)	66673 (86%) / 10603 (14%) / 399 (1%)	66425 (86%) / 10551 (14%) / 396 (1%)	248 (82%) / 52 (17%) / 3 (1%)	3.1*10^-2
MBOAT7 rs641738 C>T (CC/CT/TT), n(%)	23976 (31%) / 38247 (50%) / 14859 (19%)	23889 (31%) / 38097 (50%) / 14795 (19%)	87 (29%) / 150 (50%) / 64 (21%)	3.2*10^-1
GCKR rs1260326 C>T (CC/CT/TT), n(%)	28367 (37%) / 36977 (48%) / 12174 (16%)	28250 (37%) / 36855 (48%) / 12112 (16%)	117 (39%) / 122 (41%) / 62 (21%)	4.5*10^-1
HSD17B13 rs72613567 A>AA (TT/TAT/TATA), n(%)	40538 (52%) / 31146 (40%) / 5893 (8%)	40382 (52%) / 31024 (40%) / 5868 (8%)	156 (51%) / 122 (40%) / 25 (8%)	6.9*10^-1
Follow-up time (years), median(IQR)	9 (8.3-9.7)	9 (8.3-9.7)	5.8 (3.7-6.9)	6.5*10^-257

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, AST to platelets ratio index; BMI, body mass index; FIB-4, fibrosis-4; FLI, fatty liver index; GGT, gamma glutamyl transferase; HDL, high density lipoproteins; LDL, low density lipoproteins; NFS, NAFLD fibrosis score.

Continuous variables shown as mean (standard deviation) or median (interquartile range), if normally or not-normally distributed, respectively. Categorical variables shown as absolute numbers (percentage). P values are from generalised linear models corrected for age, sex and assessment centre.

Table 3. Baseline characteristics of the obese population according to the incidence of severe liver disease during follow-up.

term	estim.x	estim.y
age	1.06 (1.04-1.08), 1.2*10^-12	1.05 (1.03-1.07), 3.9*10^-7
malesex	2.12 (1.69-2.66), 1.0*10^-10	1.21 (0.89-1.65), 2.3*10^-1
body	1.06 (1.04-1.09), 1.2*10^-7	0.98 (0.94-1.02), 3.6*10^-1
waist	1.05 (1.04-1.06), 1.7*10^-32	1.04 (1.02-1.06), 8.5*10^-6
diab	4.5 (3.58-5.66), 3.5*10^-38	2.18 (1.55-3.05), 6.2*10^-6
ipert	2.43 (1.93-3.08), 9.9*10^-14	1.34 (1.03-1.75), 3.2*10^-2
smoke	1.6 (1.15-2.21), 4.8*10^-3	1.65 (1.17-2.31), 4.1*10^-3
above	0.86 (0.72-1.04), 1.2*10^-1	
glycated	1.47 (1.37-1.58), 8.6*10^-27	1.09 (0.96-1.23), 1.8*10^-1
pnpla3	1.62 (1.36-1.92), 4.6*10^-8	1.59 (1.33-1.9), 3.1*10^-7
pnpla3r	2.81 (1.98-3.98), 5.9*10^-9	2.62 (1.82-3.79) 2.7*10^-7
tm6sf2	1.34 (1.02-1.76), 3.4*10^-2	1.37 (1.04-1.81), 2.7*10^-2
tm6sf2r	1.95 (0.63-6.09), 2.5*10^-1	
mboat7	1.09 (0.93-1.28), 3.1*10^-1	
mboat7r	1.13 (0.86-1.49), 3.8*10^-1	
gckr	1.06 (0.9-1.24), 5.2*10^-1	
gckrr	1.39 (1.05-1.84), 2.0*10^-2	1.45 (1.09-1.93), 1.0*10^-2
hsd	1.04 (0.87-1.24), 6.9*10^-1	
hsdr	1.09 (0.73-1.65), 6.7*10^-1	

Variable associated with a p value <0.1 at univariate analysis are entered into multivariate model

	Male		Female	
term	estim.x.x	estim.y.x	estim.x.y	estim.y.y
age	1.06 (1.04-1.08), 5.8*10^-9	1.06 (1.03-1.08), 3.9*10^-5	1.06 (1.03-1.09), 3.0*10^-5	1.05 (1.02-1.08), 1.3*10^-3
malesex	NA (NA-NA), -		NA (NA-NA), -	
body	1.06 (1.03-1.09), 4.4*10^-4	0.99 (0.91-1.06), 7.2*10^-1	1.09 (1.06-1.13), 1.9*10^-8	0.98 (0.93-1.04), 4.9*10^-1
waist	1.03 (1.02-1.05), 9.3*10^-8	1.02 (1-1.05), 3.0*10^-2	1.07 (1.05-1.08), 2.2*10^-20	1.06 (1.04-1.09), 2.0*10^-6
diab	3.93 (2.96-5.21), 2.7*10^-21	2.28 (1.41-3.67), 7.1*10^-4	4.59 (3.1-6.8), 2.4*10^-14	1.97 (1.11-3.5), 2.1*10^-2
ipert	2.28 (1.69-3.09), 7.5*10^-8	1.4 (0.94-2.07), 9.5*10^-2	2.36 (1.62-3.43), 7.2*10^-6	1.24 (0.82-1.88), 3.1*10^-1
smoke	1.48 (0.99-2.21), 5.4*10^-2	1.34 (0.8-2.27), 2.7*10^-1	1.6 (0.92-2.8), 9.8*10^-2	1.73 (0.98-3.05), 6.0*10^-2
above	0.8 (0.63-1), 5.2*10^-2		0.94 (0.69-1.28), 7.0*10^-1	
glycated	1.4 (1.28-1.53), 1.2*10^-13	1.05 (0.87-1.26), 6.1*10^-1	1.54 (1.36-1.74), 4.2*10^-12	1.09 (0.88-1.36), 4.2*10^-1
pnpla3	1.65 (1.33-2.05), 5.9*10^-6	1.51 (1.17-1.96), 1.7*10^-3	1.56 (1.18-2.08), 2.0*10^-3	1.47 (1.09-1.97), 1.0*10^-2
pnpla3r	2.71 (1.74-4.23), 1.1*10^-5	2.60 (1.52-4.44), 4.8*10^-4	2.95 (1.69-5.16), 1.5*10^-4	2.80 (1.57-5), 5.0*10^-4
tm6sf2	1.4 (0.99-1.96), 5.5*10^-2	1.36 (0.91-2.03), 1.3*10^-1	1.27 (0.81-1.99), 3.0*10^-1	1.27 (0.8-2.02), 3.1*10^-1
tm6sf2r	3.27 (1.05-10.24), 4.2*10^-2	3.05 (0.75-12.32) 1.2*10^-1	-	
mboat7	1.12 (0.91-1.37), 2.9*10^-1		1.05 (0.81-1.37), 7.0*10^-1	

mboat7r	1.26 (0.9-1.77), 1.8*10^-1		0.94 (0.59-1.52), 8.1*10^-1	
gckr	0.98 (0.8-1.21), 8.8*10^-1		1.19 (0.91-1.54), 2.1*10^-1	
gckrr	1.43 (1.01-2.03), 4.4*10^-2	1.74 (1.18-2.57), 5.5*10^-3	1.33 (0.83-2.11), 2.4*10^-1	1.3 (0.81-2.09), 2.8*10^-1
hsd	1.14 (0.92-1.42), 2.4*10^-1		0.87 (0.64-1.17), 3.5*10^-1	
hsdr	1.25 (0.77-2.02), 3.7*10^-1		0.82 (0.38-1.75), 6.0*10^-1	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, AST to platelets ratio index; BMI, body mass index; FIB-4, fibrosis-4; FLI, fatty liver index; GGT, gamma glutamyl transferase; HDL, high density lipoproteins; LDL, low density lipoproteins; NFS, NAFLD fibrosis score.

Continuous variables shown as mean (standard deviation) or median (interquartile range), if normally or not-normally distributed, respectively. Categorical variables shown as absolute numbers (percentage). P values are from generalised linear models corrected for age, sex and assessment centre.

Table 4 and 5. Uni and multivariate Cox regression analysis of factors associated with incident SLD in subjects with obesity.

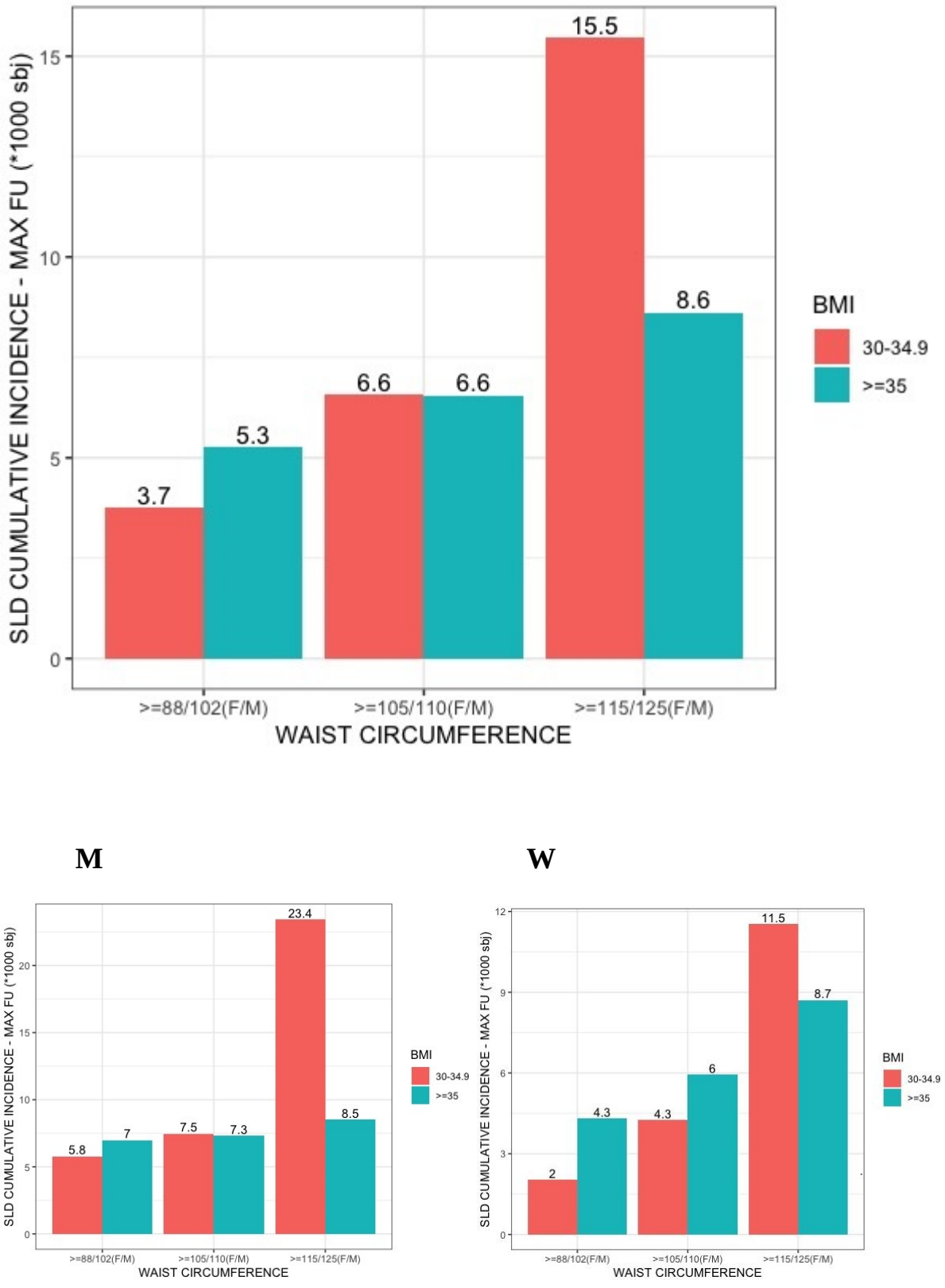
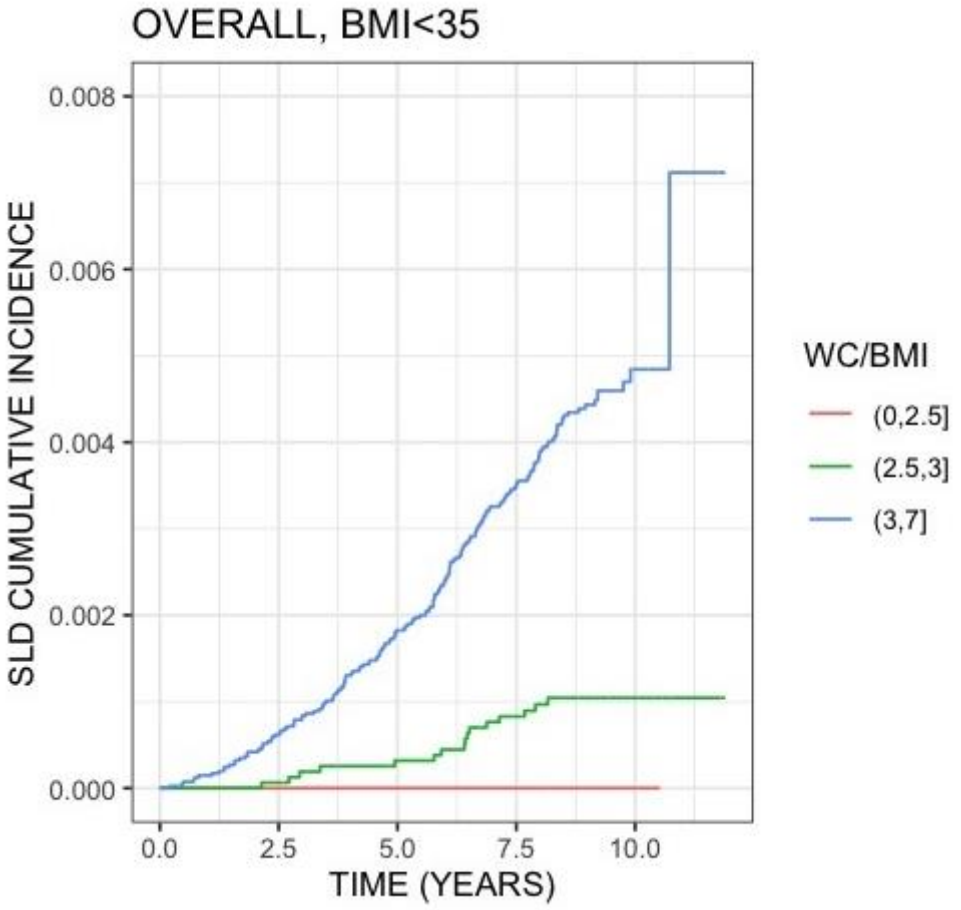
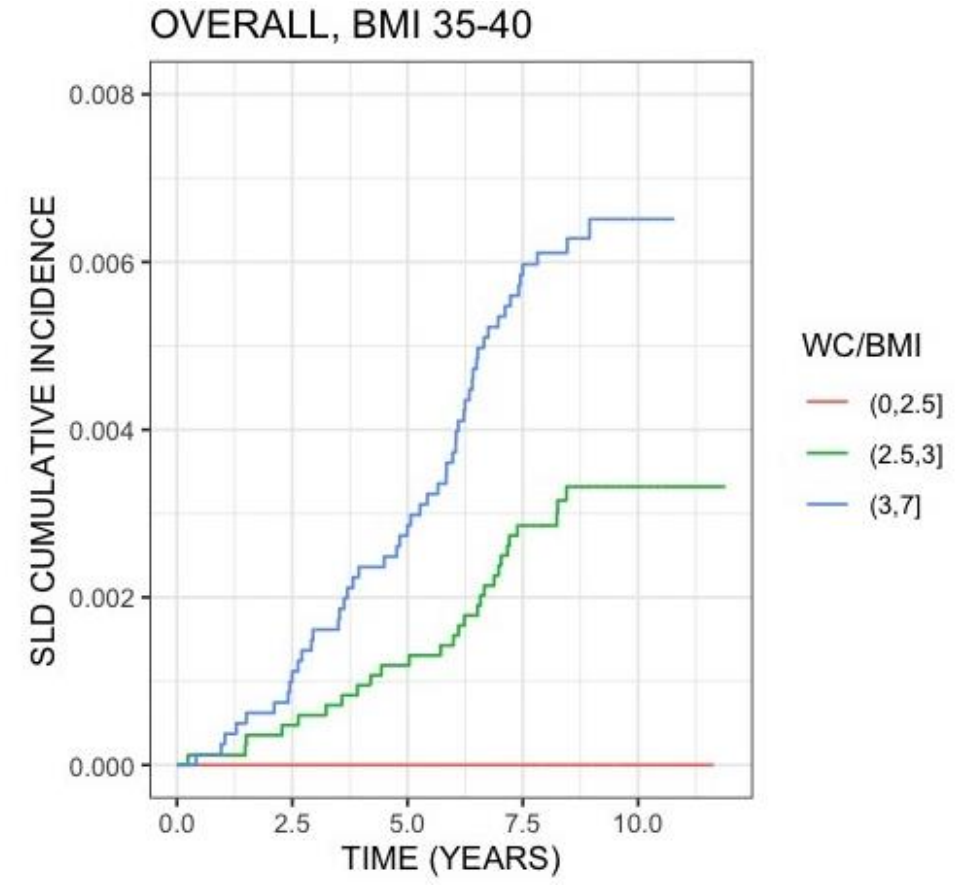


Figure 2. SLD cumulative incidence overall and stratified according to gender





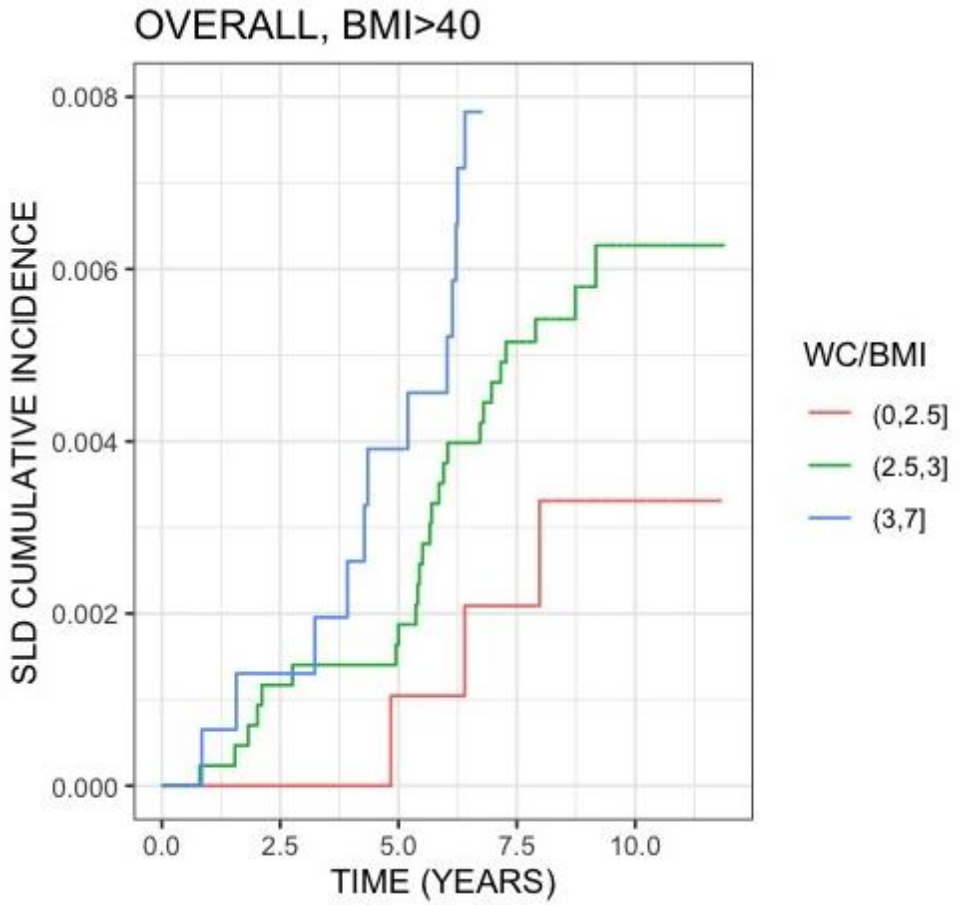


Figure 3. Overall and stratified according to BMI

CHAPTER 7: DISCUSSION AND CONCLUSIONS

In the present study, we examined for the first time risk factors for SLD in the obese individuals. We found that age, WC, type 2 diabetes and the PNPLA3 variant are independent determinants of adverse hepatic outcomes in both genders. Importantly, abdominal adiposity, measured by WC, emerged as the main mediator of SLD risk associated with BMI. Obesity and T2DM are the main risk factors for NAFLD and they are also associated with a more aggressive liver disease, i.e., the development of non-alcoholic steatohepatitis and fibrosis progression. In the present work, we showed that the incidence of SLD is higher in overweight and in obese individuals. Obesity was a significant risk factor for SLD even after adjusting for confounders, including age, sex, type 2 diabetes, hypertension and dyslipidemia. However, the association between obesity and SLD was abolished when WC was included as a covariate in the analysis, and, most notably, even when the model was adjusted only for WC. These results suggest that abdominal adiposity is the main determinant of SLD risk conveyed by obesity. When we restricted the analysis to obese subjects, the cumulative incidence of SLD was higher in those with BMI ≥35 kg/m2 as compared to those with BMI 30-34.9 kg/m2. However, in the multivariate analyses, the association with BMI was again abolished, while age, WC, type 2 diabetes, hypertension, smoking, and the PNPLA3 and TM6SF2 remained independently associated with SLD. Moreover, after stratification for genders, only age, WC, type 2 diabetes and the PNPLA3 variant were independent predictors of SLD in both genders.

Concerning genetic variants, NAFLD is a multifactorial disease whose heritability estimates has been found to range from 20% to 70%. Moreover, most of the variants which have been more consistently associated with liver fat content, i.e., PNPLA3 rs738409, TM6SF2 rs58542926, and MBOAT7 rs641738, have been subsequently associated also with development of NASH and fibrosis, suggesting that the amount of hepatic fat is a crucial driver of disease progression. The PNPLA3 polymorphism, which is recognized as the strongest determinant of interindividual and ethnicity-related differences in hepatic fat content, is here the main genetic determinant of adverse hepatic outcomes in obese subjects. In both genders, each PNPLA3 variant allele was associated with about 50% increased risk of SLD. Concerning the other polymorphisms, the association of TM6SF2 variant was abolished after stratification by gender probably due to a lack of power.

ACKNOWLEDGEMENTS

I am grateful to Professor Paolo Pozzilli for the opportunity to perform this research project; to Professor Marco Caricato for his thoughtful review and assistance; to Professor Umberto Vespasiani Gentilucci, Dr. Dario Tuccinardi and Dr. Antonio De Vincentis for their precious support and give advice.

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