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REVIEW ARTICLE

Genetics of non-alcoholic fatty liver disease and associated metabolic disorders

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Abstract Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. Whereas insulin resistance and obesity are considered major risk factors for the development and progression of NAFLD, the genetic underpinnings remain to be established. Before 2008, numerous candidate gene studies, based on prior knowledge of the pathophysiology of fatty liver, published numerous papers with conflicting results. In 2008, Romeo et al. published the first genome wide association study (GWAS), reporting the strongest genetic signal for fatty liver (*PNPLA3*, patatin-like phospholipase domain containing 3; rs738409). Most of the candidate genes and GWAS associations, however, have not been replicated and, therefore, its significance remains undetermined. Moreover, the associations between candidate genes and metabolic traits have yielded mixed results. This review summarizes the current understanding of genetic epidemiology of NAFLD and associated metabolic traits, namely, insulin resistance, adiposity and lipid disorders.

PALABRAS CLAVE
Estudios geneticos de asociación;
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Revision

Genética de la enfermedad del hígado graso no alcohólica y trastornos metabólicos asociados

Resumen La enfermedad de hígado graso no alcohólica es la enfermedad crónica del hígado más frecuente en el mundo. Considerando que la resistencia a la insulina y la obesidad son los factores de riesgo importantes para el desarrollo y progresión del hígado graso no alcohólico, las bases genéticas aún no se han establecido. Antes de 2008, se realizaron numerosos estudios de genes candidatos basándose en el conocimiento previo de la fisiopatología del hígado graso. Sin embargo, los resultados han sido contradictorios. En 2008, Romeo et al. publicó el primer estudio de asociación genómica amplia, descubriendo la señal genética más robusta para el hígado graso (PNPLA3, *patatin-like phospholipase domain containing 3*; rs738409). La mayoría de las asociaciones de genes candidatos y estudioa de asociación genómica amplia, sin embargo, no se han reproducido y, por tanto, su importancia sigue siendo indeterminada. Por otra parte,

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las asociaciones entre genes y rasgos metabólicos han arrojado resultados mixtos. Esta revisión resume el conocimiento actual de la epidemiología genética de hígado graso no alcohólica y trastornos asociados rasgos metabólicos, es decir, sensibilidad a la insulina, la adiposidad y los lípidos.

Brief epidemiology and genetic basis of non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease,[1] represents a wide spectrum of diseases characterized by the presence hepatic steatosis in the absence of significant alcohol consumption or other causes of liver disease.[2,3] The pathogenic processes leading to steatosis, steatohepatitis (NASH) and fibrosis are multifactorial and involve both environmental and genetic factors.[4]

Obesity and type 2 diabetes mellitus (T2DM)/insulin resistance are the most common risk factors for the development and progression of NAFLD.[5] A recent meta-analysis showed a direct association between waist circumference and NAFLD (odds ratio (OR) $\sim$ 1.10–1.13 per 1 cm increase in waist circumference) and between intra-abdominal fat and NAFLD (OR = 1.50 per 1 mm increase in visceral fat thickness).[6] In addition, between 47% and 71% of NAFLD patients have metabolic syndrome,[7] and conversely, the presence of metabolic syndrome is a strong predictor for NAFLD.[8] Consequently, NAFLD is considered the hepatic component of the metabolic syndrome.[9] As expected, patients with T2DM have shown a prevalence of NAFLD ranging from 40% to 80%,[10,11] and the presence of T2DM is associated with more severe liver disease.[12] Since both obesity and T2DM continue to rise, it is expected that NAFLD will reach epidemic proportions worldwide. The prevalence of ultrasound-defined NAFLD ranges from 14 to 45%.[13] In Spain, Caballeria et al., in a recent cross-sectional study, showed a prevalence of 26% in individuals aged 15 and 85 years randomly selected from 25 Primary Healthcare Centers,[14] consistent with previous estimates.

A genetic underpinning for NAFLD has been suggested by familial aggregation studies,[15,16] heritability studies,[17,18] candidate gene studies,[4] genome-wide scans[19–22] and expression studies.[23–27] The presence of a genetic basis in NAFLD is important in order not only to identify, individuals at risk, but also to dissect NAFLD pathogenesis and the development of new therapies.

The ultimate goal of this review is to provide the most recent studies in the genetic epidemiology of NAFLD and its association with metabolic traits, namely, adiposity/obesity, insulin resistance, and dyslipidemia. The review is structured in two parts: first, the author will provide some background concepts in genetic epidemiology and the current understanding of NAFLD pathophysiology; and, secondly, the reviewer will show the most up-to-date findings in humans in candidate gene studies and genome wide association studies (GWAS) and their association with metabolic traits. This paper, however, will not assess other forms of fatty liver disease, expression studies or animal studies.

Concepts in genetic epidemiology of NAFLD

NAFLD is considered a complex disease, that is, there is no simple Mendelian pattern of single-gene inheritance such as maturity onset diabetes of the young. In NAFLD, multiple genes, environmental factors, age effects, and their interactions, may be involved.[28]

Genetic epidemiology studies can be divided in two broad categories: (a) according to the relationships between participants (family-based *versus* non-related or population-based); and, (b) according to the knowledge of the genetic marker(s) used (hypothesis-driven or candidate gene study *versus* hypothesis free or genome wide association study). A candidate gene is defined as a gene in which its product is considered to play a role in disease pathogenesis.[29] It is important to bear in mind that all studies provide key information and are not mutually exclusive. For instance, family-based studies are typically the first step in determining whether a condition is genetic in nature, the model of inheritance, and to understand the proportion of phenotypic variance due to shared genetic factors (heritability). Family-based studies may use *linkage analysis* to identify the location of a major gene or, association analyses such as the transmission-disequilibrium test, candidate gene approach and a genome-wide association analysis.

Population-based studies, on the other hand, are the mainstream of studies in NAFLD and, until recently, the candidate gene study was the most common design using the ''two hit'' hypothesis as a framework.[30]

Both candidate gene and GWAS rely on the statistical association of genetic markers and the disease (phenotype) of interest. The most commonly used genetic markers are the single nucleotide polymorphisms or SNPs, defined as a change in a single base pair of the DNA common in more than 1% of the population.[31]

The major problem in both candidate gene and GWAS is the presence of false negative and false positive associations. The interpretation of a negative study in genetics is challenging, but most of the time, the problem is a lack of statistical power, that is, true associations are not detected in the study. Consequently, power calculation will provide a robust answer whether the association is indeed possible or not. In GWAS, where there is no hypothesis behind the statistical analysis, the problem we encounter is the opposite,

and we commonly find spurious associations due to chance, even after statistical correction for multiple comparisons. As a result, most of the genetics journals in the same paper need to replicate newly associated genes in different, independent samples (known as 'replication'). The final step is gene fine sequencing to understand what the mechanism is for the association of this particular gene, and use of animal models (knock-outs) and in vitro studies to characterize the metabolic effect of that particular mutation.

Current understanding of NAFLD pathogenesis

The key pathological finding in fatty liver disease is the accumulation of triglycerides in the hepatocytes due to a disproportion between triglycerides acquisition and removal.[32,33] The key component of triglyceride is the long-chain fatty acid. The major routes of free fatty acids (FFA) are (a) dietary intake; (b) lipolysis from fat reservoirs, and (c) *de novo* lipogenesis. Animal and human inherited diseases have shown increased hepatic steatosis with increased triglyceride dietary intake, increased lipolysis from peripheral tissues, increased *de novo* lipogenesis, or prevention of triglyceride removal from the liver (i.e. decreased efflux or oxidation).[32] The exact contributions of each pathway to the development of hepatic steatosis have been studied by Donnelly et al.,[34] who showed that 59% of hepatic fat derived from circulating FFAs (mainly lipolysis), 26% from *de novo* lipogenesis and 15% from diet. The latter mechanism is particularly interesting in the context of obesity and frequent consumption of refined sugars. Increased glucose intake has been associated with hepatic steatosis due to the *de novo* synthesis of free fatty acids by increased insulin (up-regulator of the transcription factor sterol regulatory element-binding protein-1c, SREBP-1c), availability of substrate and the activation of the transcription factor carbohydrate responsive element-binding protein (ChREBP), involved in the expression of lipogenic genes.[32,33]

Insulin resistance is an essential key etiopathogenic factor in the development and progression of fatty liver. Whether insulin resistance is a cause of hepatic steatosis, or vice versa, is a matter of debate and has been reviewed recently.[32] According to Cohen et al.,[32] current evidence suggests that insulin resistance comes *first* and leads to hepatic steatosis, as shown by animal models and human diseases where hepatic steatosis is not associated with insulin resistance and, conversely, human diseases with inherited insulin resistance lead to hepatic steatosis (e.g. *AKT2* mutation). Opposite views, however, suggested that hepatic steatosis, possibly originated by impaired mitochondrial β-oxidation of fatty acids, leads to hepatic insulin resistance which, in turn, increases *de novo* fatty acid synthesis, impairs gluconeogenesis, hepatic glucose uptake, transport of lipoproteins perpetuating increase peripheral insulin resistance.[13,33]

A ''two-hit model'' for the development of steatohepatitis was proposed by Day and James in 1998. In their view, the first step was the accumulation of free fatty acids in the liver, and, from there, further insults induced inflammation, fibrosis and, eventually, cirrhosis.[30] Nevertheless, Dr. Day's group, based on current evidence from animal models, updated their own hypothesis and suggested that ''steatosis may be early adaptive response to hepatocyte stress through which potentially lipotoxic FFAs are partitioned into relatively stable intracellular triglyceride stores''.[35] NAFLD is now seen ''as a combination of effects of several fundamental biochemical and immunological mechanisms of liver injury rather than adhering to a sequential 'two-hit' paradigm''.[35]

In summary, based on Day et al., ''insulin resistance promotes increased hepatic FFA flux (lipolysis, diet and lipogenesis) leading to hepatic steatosis, and is driven by (1) direct hepatocyte lipotoxicity, (2) hepatocellular oxidative stress secondary to free radicals produced during b- and -FFA oxidation, (3) endotoxin/TLR4-induced inflammation, (4) cytokine release, and (5) endoplasmic reticulum (ER) stress'', finally leading to inflammation, cellular damage, and progression to cirrhosis.

A systematic approach to reviewing the genetic epidemiology of NAFLD

In order to identify most of the available published literature on the genetics of NAFLD, the author performed a systematic search using PUBMED, by applying the following engine: (polymorphism OR SNP) AND (''steatosis'' OR ''steatohepatitis'' OR NAFLD OR NASH) without language restriction and up to July 26th, 2011. Studies were included and analyzed if they were performed on humans. The search yielded 224 references, of which 60 corresponded to candidate gene studies and are summarized in Table 1 , and three genome wide association studies (Table 2). This search engine did not use the more 'non-English language friendly' database EMBASE, and was limited by the published papers (risk of publication bias since negative studies usually remain unpublished). Genes (or nearby genes) are named using the HUGO Gene Nomenclature Committee (HGNC) and reported identification number.[36]

Candidate genes studies in human NAFLD: hepatic lipid metabolism, insulin resistance and oxidative stress

Numerous candidate gene studies applying this 'multiple-hit' hypothesis, have studied the effects of genes on NAFLD presence and progression. The reviewer has simplified the previous hypothesis into the following mechanisms (not mutually exclusive): genes involved in hepatic lipid metabolism (synthesis, storage, export, oxidation), genes implicated in insulin signaling (insulin resistance), and finally, genes involved in oxidative stress and inflammation (and therefore, most likely involved in progression to cirrhosis) (Table 1). The reviewer included, when appropriate, other data from different metabolic traits to illustrate the direction of association for a particular gene (in parenthesis). Interestingly, the strongest signals for other well-known risk factors, such as obesity or diabetes (e.g. *FTO*, *TCF7L2*) did not associate with fatty liver disease, suggesting that fatty liver disease, on its own, has other genetic underpinnings.

Table 1 Candidate gene studies in non-alcoholic fatty liver disease and its association with metabolic traits.

Gene (HGNC), SNP	Fatty liver	Inflammation/ fibrosis	Insulin resistance (*)	Adiposity (*)	Lipids (*)
Hepatic lipid metabolism					
PEMT (8830),[43–46] phosphatidylethanolamine N-methyltransferase; rs7946	⌀↑↑	↑	⌀	⌀	↑
MTTP (7467),[47–51] microsomal triglyceride transfer protein; rs1800591	⌀⌀	⌀↑↑↑↑	⌀↑↑	⌀↑ (↓[52])	⌀↑↑
APOC3 (610),[53,54] apolipoprotein C-III; rs2854116/2854117	⌀↑		↑⌀	⌀⌀	↑⌀(↑[55])
FABP2 (2556),[56,57] fatty acid binding protein 2, intestinal; rs1799883	↑		⌀(↑[58])	⌀(⌀[59])	⌀(↑[60])
DGAT (2843),[61] acyl-CoA:diacylglycerol acyltransferase; rs1944438	⌀		⌀	⌀ (⌀[62])	(↑[63])
ACSL4 (3571),[64] acyl-CoA synthetase long-chain family member 4; rs7887981	↑			↑	↑
ADRB3 (288),[65] adrenergic, beta-3-, receptor; rs4994	↑	↑	↑(↑[66])	↑(⌀[67])	↑(↑[68])
ADRB2 (286),[69] adrenergic, beta-2-, receptor, surface; rs1042714	↑		⌀(⌀[70])	⌀(⌀[71])	⌀
LIPC (6619),[43,72] hepatic lipase; rs1800588	↑↑			↑	(↑[73,74,41,75])
APOE (613),[76–79] apolipoprotein E; N/A	⌀↑↓↓	↑	(↑[80])	↑	↑↑(⌀[80]↑[41])
CLOCK (2082),[81] clock homolog (mouse); rs11932595	↑	↑	↑(↑[82])		
Insulin sensitivity					
ENPP1 (3356),[47,83] ectonucleotide pyrophosphatase/phosphodiesterase 1 or PC-1; rs1044498	⌀⌀	⌀⌀↑	⌀⌀(↑[84,85])	⌀⌀(↑[86])	⌀⌀
IRS1 (6125),[83] insulin receptor substrate 1; rs1801278	⌀	⌀⌀	⌀(⌀[87,88])	⌀	⌀
ADIPOQ (13633),[89–92] adiponectin, C1Q and collagen domain containing; rs2241766	⌀⌀⌀↑↑	⌀↑	↑↑(↑[93–95])	⌀↑(↑[96])	↑↑↑
Insulin sensitivity					
PPARA (9232),[97,98] peroxisome proliferator-activated receptor alpha, rs1800206	⌀↑	⌀⌀			
PPARG (9236),[43] peroxisome proliferative activated receptor, gamma; rs3856806	⌀				

Table 1 (*Continued*)

Gene (HGNC), SNP	Fatty liver	Inflammation/ fibrosis	Insulin resistance (*)	Adiposity (*)	Lipids (*)
PPARG, [97,99] rs1801282	⌀⌀	⌀⌀⌀⌀	↓ ↓ (↓[100–104])	↑(↑[103])	↑ (↑[105])
PPARGC1A (9237),[43] peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; rs8192687	⌀		(↑[106])	(⌀[106])	
TCF7L2 (11641),[107] transcription factor 7-like 2 (T-cell specific, HMG-box); rs7903146	↑	↑↑	↑(↑[108–111,102])		↑
GCKR (4196),[112] glucokinase (hexokinase 4) regulator; rs780094	↑		(↑[113,114,110,115–117])		↑(↑[113,114,110,115–117])
MC4R (6932),[118] melanocortin-4 receptor; rs17782313	⌀		⌀	↑(↓[119–124])	⌀
SPINK-1 (11244),[125] Serine protease inhibitor Kazal-1; N/A	⌀				
LEPR (6554),[126] leptin receptor gene; N/A	↑		(↑[127])	(⌀[128–130])	
LEP (6553),[43] leptin; rs7799039	⌀			(⌀[96,131])	
Genes influencing generation of reactive oxidant species, or cytokine genes					
TNF (11892),[43,92,132–135] Tumor necrosis factor-alpha; rs180062	⌀⌀⌀	↑↑⌀⌀⌀	⌀⌀⌀(↑[136])	⌀⌀(↑[136])	⌀⌀
IL-6 (6018),[47] rs1800795	↑	↑	↑(⌀[137])	⌀(↑[138])	⌀
*CD14 (*1628),[139] rs2569190	⌀	↑	(↑[140])		
SOD2 (11180),[50,51,141] superoxide dismutase 2, mitochondrial; rs4880	↑	⌀↑↑↑			
HFE (4886),[37] hemochromatosis; rs1800562, rs1799945	⌀	⌀	⌀(↑[38])	⌀	⌀
ABCB11 (42),[142] ATP-binding cassette, subfamily B, member 11; rs2287622	⌀	⌀⌀	(↑[38])		
CFTR/MRP (53),[143] ATP-binding cassette, sub-family C, member 2; rs17222723 rs8187710	↑	⌀	↑	↑	

Parenthesis denotes studies done with metabolic traits but not with liver steatosis

Table 2 Genome wide association studies in nonalcoholic fatty liver disease and associated metabolic traits.

Gene (HGNC)	SNP	Fatty liver	Inflammation/ fibrosis	Insulin resistance (*)	Adiposity (*)	Lipids (*)
PNPLA3 (18590), patatin-like phospholipase domain containing 3[20,21]	rs738409	↑↑		∅(↑[144])	∅	↑∅[42]
PNPLA3 (18590), patatin-like phospholipase domain containing 3[20]	rs6006460	↓				
FDFT1 (3629),[19] farnesyl diphosphate farnesyl transferase 1	rs2645424		↑			
COL13A1 (2190),[19] collagen, type XIII, alpha 1	rs1227756		↑			
PDGFA (8799),[19] platelet-derived growth factor alpha polypeptide	rs343064		↑			
LTBP3 (6716),[19] latent transforming growth factor beta binding protein 3	rs1227756		↑			
EFCAB4B (28657),[19] EF-hand calcium binding domain 4B	rs887304		↑			
NCAN (2465),[21] neurocan	rs2228603	↑				(↑[41,145])
LYPLAL1 (20440),[21] lysophospholipase-like 1	rs12137855	↑			(↑[146–149])	
GCKR (4196),[21] glucokinase regulatory protein	rs780094	↑		(↑[113,114,110,115–117])		(↑[113,114,110,115–117])
PPP1R3B (14942),[21] protein phosphatase 1, regulatory subunit 3b	rs4240624	↑		(↑[150])		(↑[151,152])

Parenthesis denotes studies done with metabolic traits but not with liver steatosis

Hepatic lipid metabolism

The alteration in the management of triglycerides handling would likely lead to the development of hepatic steatosis. One of the most promising candidate genes was the microsomal triglyceride transfer protein (*MTTP*, 7467) central in lipoprotein role assembly and its inhibition in familial hypercholesterolemia was shown to increase liver steatosis. Unfortunately, this review showed inconsistent association with fatty liver disease and a marginal effect on related metabolic traits, suggesting that it is not clearly implicated in the development of fatty liver disease, or that the studies lack power to detect any differences. Other natural metabolic pathways in lipid metabolism, such as lipoprotein metabolism and hepatic lipase activity, have yielded similar inconsistent associations with hepatic steatosis (Table 1).

Insulin sensitivity

NAFLD has been considered the liver component of the metabolic syndrome and, therefore, insulin resistance is supposed to be a key etiopathogenetic factor. Adiponectin is a cytokine with insulin-sensitizing, antifibrogenic and anti-inflammatory roles and, in histology, hypoadiponectinemia, have been associated with advanced NAFLD. As shown in Table 1, the association with steatosis is inconsistent with adiponectin polymorphisms.

Iron overload, commonly associated with progression of liver disease, insulin resistance and oxidative stress, have been suggested to play a role in the development and progression of NAFLD. Our recent meta-analysis has shown that the hemochromatosis gene (*HFE*) has no association with the NAFLD or advanced NASH.[37] Finally, other appealing genes such as *PPARG* or *TCF7L2* have been associated with either liver steatosis and metabolic traits, however, the bulk of the literature shows minimal association, and warrants more research. Recently, a GWAS consortium have shown that, except for the *GCKR*, none of the reported candidate genes in NALFD has been associated with levels of A1c highlighting that fat accumulation may represent a different type of individual with diabetes.[38]

Oxidative stress

The association with polymorphisms associated with oxidative stress would help in understanding the progression of NAFLD to NASH/cirrhosis and, through oxidation of lipoproteins, its association with cardiovascular disease. Up to date, there is no strong evidence to pinpoint a particular polymorphism associated with progression of liver disease or associated with oxidation of lipoproteins (Table 1).

Genome wide association studies in fatty liver disease

In 2008, Romeo et al. were the first to apply the GWAS method using a phenotype based on magnetic resonance spectroscopy.[20] They studied 9,299 non-synonymous sequence variations and identified a missense mutation [Ile148 → Met148 (I148M)] in a patatin-like phospholipase domain-containing (PNPLA) 3 gene *PNPLA3* (HCNG: 18590). *PNPLA3*, highly expressed in adipose tissue and liver, is regulated by insulin through a signaling cascade that includes LXR and SREBP-1c[32] and, therefore, increased with feeding

in animal studies.[39] This mutation is, by far, the strongest genetic signal up to date and showed a increased odds ratio for fatty liver of 3.26 in the original report.[20] In addition, the PNPLA3 gene could also be responsible for the difference in prevalence of fatty liver disease between ethnic groups. For instance, Mexican-Americans have a higher prevalence of the high risk allele, whereas African Americans, where fatty liver is known to be less frequent, had a protective variation for such.[20] Further studies confirmed the association between this gene and the presence of fatty liver disease, including GWAS with liver enzymes[22] and multiple case-control studies[40] (Table 2).

Chalasani et al.[19] described, in 236 white female biopsy-proven NAFLD patients, five new genetic variants associated with inflammation and fibrosis: a variant in the *FDFT1* (farnesyl diphosphate farnesyl transferase 1, HGNCI 3629) was correlated with NAFLD; in addition, they found an association with lobular inflammation for the collagen gene *COL13A1* (collagen, type XIII, alpha 1, HGNC 2190), a SNP near the *PDGFA* gene (platelet-derived growth factor alpha polypeptide, HGNC 8799); the *LTBP3* (latent transforming growth factor beta-binding protein 3, HGNC 6716), and the *EFCAB4B* (EF-hand calcium binding domain 4B, HGNC: 28657, Table 2).

Most recently, we showed, in the Genetics of Obesity-related Liver Disease (GOLD) Consortium, four additional genetic variants in addition to the *PNPLA3* (HGNC: 18590), namely, *NCAN* (neurocan, HGNC: 2465), *LYPLAL1* (lysophospholipase-like 1, HGNC: 20440); *GCKR* (glucokinase regulatory protein, HGNC: 4196); and the *PPP1R3B* (protein phosphatase 1, regulatory subunit 3b, HGNC: 14942), Table 2. *GCKR* and *PPP1R3B* are key enzymes in *de novo* lipogenesis from glucose and *LYPLAL1*-related protein, and make them plausible for having a role in consecutive steps in triglyceride breakdown. The role of *NCAN* remains to be determined and has been associated with lipid traits.[41] Interestingly, we found that *PNPLA3* had a modest role in lipid metabolism, suggesting that these genes, if they are involved in lipid metabolism, exert their liver effects within the liver through different mechanistic pathways than those observed by conventional laboratory lipid measurement. In addition, *PNPLA3* has been shown to have a null effect on insulin sensitivity, or, surprisingly an insulin-sensitizing effect in obese carriers,[42] consistent with the current hypothesis that fat in the liver does not lead to insulin resistance and may be a protective stage. A recent GWAS showed, however, that A1c levels were associated with GCKR mutations and, therefore, this would be a common link in why patients with NAFLD have increased levels of A1c compared to the general population.[38]

Future directions: candidate gene studies

Old and new genes have been reported in association with NAFLD, and all show the same problem: the need for replication. The major caveat of candidate gene studies is the lack of power. For instance, assuming a genetic risk ratio of 1.5 for a given polymorphism (high for most of the replicated genetic studies), and a allele frequency of about 20%, more than 400 patients and controls are required to give a study 90% power to detect a significant effect at the 5% level.[29] Day[29] and Bataller[31] provide two outstanding reviews to guide the reader in the design of high quality candidate gene studies, step-by-step.

On the other hand, if the researcher is willing to invest his/her time to study new candidate genes, then Day proposes to find them by reviewing: ''(1) gene product considered to play a role in the disease; (2) gene is known to be mutated in a familial form of the disease, (3) gene knockout/overexpression in animal models influences disease development; (4) gene lies in a chromosomal region associated with disease in a linkage study; (5) gene expression is altered in microarray studies of tissue from patients with disease; (6) gene is identified in a phenotype-driven mouse mutagenesis study''[29]; and (7) gene identified in genome-wide association studies.

In conclusion, genes play a role in the development and progression of NAFLD; *PNPLA3* is the strongest signal up to date, but there are other numerous genes that have been described but not formally replicated. Candidate genes for metabolic traits have yielded conflicting results in their association with NAFLD. Therefore, the future of genetic epidemiology will require replication and, ultimately, expression studies and animal models to know the molecular role of that particular genetic variant. The understanding of genetic determinants of NAFLD will help to identify individuals at risk and, potentially, new therapies to treat the most common chronic liver disease in the world.

Conflict of interests

The author declares no conflict of interests.

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References

1. Lazo M, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. Semin Liver Dis. 2008;28:339–50.
2. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. Semin Liver Dis. 2001;21:3–16.
3. Sanyal AJ. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology. 2002;123:1705–25.
4. Daly AK, Ballestri S, Carulli L, Loria P, Day CP. Genetic determinants of susceptibility and severity in nonalcoholic fatty liver disease. Expert Rev Gastroenterol Hepatol. 2011;5:253–63.
5. Moreno-Sanchez D. [Epidemiology and natural history of primary nonalcoholic fatty liver disease]. Gastroenterol Hepatol. 2006;29:244–54.
6. Jakobsen MU, Berentzen T, Sorensen TI, Overvad K. Abdominal obesity and fatty liver. Epidemiol Rev. 2007;29:77–87.
7. Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, et al. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. Hepatology. 2002;35:367–72.

8. Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, et al. The metabolic syndrome as a predictor of non-alcoholic fatty liver disease. Ann Intern Med. 2005;143:722–8.
9. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes. 2001;50:1844–50.
10. Angelico F, Del BM, Conti R, Francioso S, Feole K, Fiorello S, et al. Insulin resistance, the metabolic syndrome, and nonalcoholic fatty liver disease. J Clin Endocrinol Metab. 2005;90:1578–82.
11. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2007;30:1212–8.
12. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology. 1999;30:1356–62.
13. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology. 2006;432Suppl1:S99–112.
14. Caballeria L, Pera G, Auladell MA, Toran P, Munoz L, Miranda D, et al. Prevalence and factors associated with the presence of nonalcoholic fatty liver disease in an adult population in Spain. Eur J Gastroenterol Hepatol. 2010;22:24–32.
15. Abdelmalek MF, Liu C, Shuster J, Nelson DR, Asal NR. Familial aggregation of insulin resistance in first-degree relatives of patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2006;4:1162–9.
16. Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. Am J Gastroenterol. 2001;96:2957–61.
17. Brouwers MC, Van Greevenbroek MM, Cantor RM. Heritability of nonalcoholic fatty liver disease. Gastroenterology. 2009;137:1536.
18. Schwimmer JB, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology. 2009;136:1585–92.
19. Chalasani N, Guo X, Loomba R, Goodarzi MO, Haritunians T, Kwon S, et al. Genome-wide association study identifies variants associated with histologic features of nonalcoholic fatty liver disease. Gastroenterology. 2010;139:1567–76.
20. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008;40:1461–5.
21. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet. 2011;7:e1001324.
22. Yuan X, Waterworth D, Perry JR, Lim N, Song K, Chambers JC, et al. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. Am J Hum Genet. 2008;83:520–8.
23. Caballero F, Fernandez A, De Lacy AM, Fernandez-Checa JC, Caballeria J, Garcia-Ruiz C. Enhanced free cholesterol SREBP-2 and StAR expression in human NASH. J Hepatol. 2009;50:789–96.
24. Guillen N, Navarro MA, Arnal C, Noone E, Rbones-Mainar JM, Acin S, et al. Microarray analysis of hepatic gene expression identifies new genes involved in steatotic liver. Physiol Genomics. 2009;37:187–98.
25. Miquilena-Colina ME, Lima-Cabello E, Sanchez-Campos S, Garcia-Mediavilla MV, Fernandez-Bermejo M, Lozano-Rodriguez T, et al. Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C. Gut. 2011;60:1394–402.
26. Yoneda M, Endo H, Mawatari H, Nozaki Y, Fujita K, Akiyama T, et al. Gene expression profiling of non-alcoholic steatohepatitis using gene set enrichment analysis. Hepatol Res. 2008;38:1204–12.
27. Younossi ZM, Baranova A, Ziegler K, Del GL, Schlauch K, Born TL, et al. A genomic and proteomic study of the spectrum of nonalcoholic fatty liver disease. Hepatology. 2005;42:665–74.
28. Thomas DT. Statistical methods in genetic epidemiology. 1st ed. New York: Oxford University Press; 2004.
29. Day CP. Genetic studies to identify hepatic fibrosis genes and SNPs in human populations. Methods Mol Med. 2005;117:315–31.
30. Day CP, James OF. Steatohepatitis: a tale of two ''hits''? Gastroenterology. 1998;114:842–5.
31. Bataller R, North KE, Brenner DA. Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. Hepatology. 2003;37:493–503.
32. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. Science. 2011;332:1519–23.
33. Sanyal AJ. Mechanisms of disease: pathogenesis of nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol. 2005;2:46–53.
34. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115:1343–51.
35. Anstee QM, Daly AK, Day CP. Genetics of alcoholic and nonalcoholic Fatty liver disease. Semin Liver Dis. 2011;31:128–46.
36. HUGO Gene Nomenclature Committee at the European Bioinformatics Institute. HUGO Gene Nomenclature Committee (HGNC) [consultado 28 Jul 2011]. Disponible en: http://www genenames org.
37. Hernaez R, Yeung E, Clark JM, Kowdley KV, Brancati FL, Kao WH. Hemochromatosis gene and nonalcoholic fatty liver disease: A systematic review and meta-analysis. J Hepatol. 2011;55:1079–85.
38. Soranzo N, Sanna S, Wheeler E, Gieger C, Radke D, Dupuis J, et al. Common variants at 10 genomic loci influence hemoglobin A(C) levels via glycemic and nonglycemic pathways. Diabetes. 2010;59:3229–39.
39. Huang Y, He S, Li JZ, Seo YK, Osborne TF, Cohen JC, et al. A feed-forward loop amplifies nutritional regulation of PNPLA3. Proc Natl Acad Sci U S A. 2010;107:7892–7.
40. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of non-alcoholic fatty liver disease. Hepatology. 2011;53:1883–94.
41. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet. 2008;40:161–9.
42. Kantartzis K, Peter A, Machicao F, Machann J, Wagner S, Konigsrainer I, et al. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. Diabetes. 2009;58:2616–23.
43. Zhou YJ, Li YY, Nie YQ, Yang H, Zhan Q, Huang J, et al. Influence of polygenetic polymorphisms on the susceptibility to non-alcoholic fatty liver disease of Chinese people. J Gastroenterol Hepatol. 2010;25:772–7.
44. Jun DW, Han JH, Jang EC, Kim SH, Kim SH, Jo YJ, et al. Polymorphisms of microsomal triglyceride transfer protein gene and phosphatidylethanolamine N-methyltransferase gene in alcoholic and nonalcoholic fatty liver disease in Koreans. Eur J Gastroenterol Hepatol. 2009;21:667–72.
45. Dong H, Wang J, Li C, Hirose A, Nozaki Y, Takahashi M, et al. The phosphatidylethanolamine N-methyltransferase gene V175M single nucleotide polymorphism confers the susceptibility to NASH in Japanese population. J Hepatol. 2007;46:915–20.

46. Song J, Da Costa KA, Fischer LM, Kohlmeier M, Kwock L, Wang S, et al. Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). FASEB J. 2005;19:1266–71.
47. Carulli L, Canedi I, Rondinella S, Lombardini S, Ganazzi D, Fargion S, et al. Genetic polymorphisms in non-alcoholic fatty liver disease: interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis. Dig Liver Dis. 2009;41:823–8.
48. Musso G, Gambino R, Cassader M. Lipoprotein metabolism mediates the association of MTP polymorphism with beta-cell dysfunction in healthy subjects and in nondiabetic normolipidemic patients with nonalcoholic steatohepatitis. J Nutr Biochem. 2010;21:834–40.
49. Oliveira CP, Stefano JT, Cavaleiro AM, Zanella Fortes MA, Vieira SM, Rodrigues LV, et al. Association of polymorphisms of glutamate-cystein ligase and microsomal triglyceride transfer protein genes in non-alcoholic fatty liver disease. J Gastroenterol Hepatol. 2010;25:357–61.
50. El-Koofy NM, El-Karaksy HM, Mandour IM, Anwar GM, El-Raziky MS, El-Hennawy AM. Genetic polymorphisms in non-alcoholic fatty liver disease in obese Egyptian children. Saudi J Gastroenterol. 2011;17:265–70.
51. Namikawa C, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, et al. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. J Hepatol. 2004;40:781–6.
52. Bohme M, Grallert H, Fischer A, Gieger C, Nitz I, Heid I, et al. MTTP variants and body mass index, waist circumference and serum cholesterol level: Association analyses in 7582 participants of the KORA study cohort. Mol Genet Metab. 2008;95:229–32.
53. Petersen KF, Dufour S, Hariri A, Nelson-Williams C, Foo JN, Zhang XM, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med. 2010;362:1082–9.
54. Kozlitina J, Boerwinkle E, Cohen JC, Hobbs HH. Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. Hepatology. 2011;53:467–74.
55. Corella D, Guillen M, Saiz C, Portoles O, Sabater A, Folch J, et al. Associations of LPL and APOC3 gene polymorphisms on plasma lipids in a Mediterranean population: interaction with tobacco smoking and the APOE locus. J Lipid Res. 2002;43:416–27.
56. Peng X, Zhang L, Wang Q, Cui X. [Study on the relationship between FABP2 Ala54Thr polymorphism and the risk of non-alcoholic fatty liver diseases]. Wei Sheng Yan Jiu. 2009;38:401–4.
57. Aller R, De Luis DA, Fernandez L, Calle F, Velayos B, Izaola O, et al. Influence of Ala54Thr polymorphism of fatty acid-binding protein 2 on histological alterations and insulin resistance of non alcoholic fatty liver disease. Eur Rev Med Pharmacol Sci. 2009;13:357–64.
58. Zhao T, Zhao J, Yang W. Association of the fatty acid-binding protein 2 gene Ala54Thr polymorphism with insulin resistance and blood glucose: a meta-analysis in 13451 subjects. Diabetes Metab Res Rev. 2010;26:357–64.
59. Zhao T, Zhao J, Lv J, Nzekebaloudou M. Meta-analysis on the effect of the Ala54Thr polymorphism of the fatty acid-binding protein 2 gene on body mass index. Nutr Metab Cardiovasc Dis. 2011;21:823–9.
60. Zhao T, Nzekebaloudou M, Lv J. Ala54Thr polymorphism of fatty acid-binding protein 2 gene and fasting blood lipids: a meta-analysis. Atherosclerosis. 2010;210:461–7.
61. Kantartzis K, Machicao F, Machann J, Schick F, Fritsche A, Haring HU, et al. The DGAT2 gene is a candidate for the dissociation between fatty liver and insulin resistance in humans. Clin Sci (Lond). 2009;116:531–7.
62. Coudreau SK, Tounian P, Bonhomme G, Froguel P, Girardet JP, Guy-Grand B, et al. Role of the DGAT gene C79T single-nucleotide polymorphism in French obese subjects. Obes Res. 2003;11:1163–7.
63. Ludwig EH, Mahley RW, Palaoglu E, Ozbayrakci S, Balestra ME, Borecki IB, et al. DGAT1 promoter polymorphism associated with alterations in body mass index, high density lipoprotein levels and blood pressure in Turkish women. Clin Genet. 2002;62:68–73.
64. Kotronen A, Yki-Jarvinen H, Aminoff A, Bergholm R, Pietilainen KH, Westerbacka J, et al. Genetic variation in the ADIPOR2 gene is associated with liver fat content and its surrogate markers in three independent cohorts. Eur J Endocrinol. 2009;160:593–602.
65. Nozaki Y, Saibara T, Nemoto Y, Ono M, Akisawa N, Iwasaki S, et al. Polymorphisms of interleukin-1 beta and beta 3-adrenergic receptor in Japanese patients with nonalcoholic steatohepatitis. Alcohol Clin Exp Res. 2004;28(8 Suppl). Proceedings: 106S-110S.
66. Morcillo S, Cardona F, Rojo-Martinez G, Almaraz MC, Esteva I, Ruiz-De-Adana MS, et al. Effect of the combination of the variants -75G/A APOA1 and Trp64Arg ADRB3 on the risk of type 2 diabetes (DM2). Clin Endocrinol (Oxf). 2008;68:102–7.
67. Kurokawa N, Young EH, Oka Y, Satoh H, Wareham NJ, Sandhu MS, et al. The ADRB3 Trp64Arg variant and BMI: a meta-analysis of 44 833 individuals. Int J Obes (Lond). 2008;32:1240–9.
68. Corella D, Guillen M, Portoles O, Sorli JV, Alonso V, Folch J, et al. Gender specific associations of the Trp64Arg mutation in the beta3-adrenergic receptor gene with obesity-related phenotypes in a Mediterranean population: interaction with a common lipoprotein lipase gene variation. J Intern Med. 2001;250:348–60.
69. Iwamoto N, Ogawa Y, Kajihara S, Hisatomi A, Yasutake T, Yoshimura T, et al. Gln27Glu beta2-adrenergic receptor variant is associated with hypertriglyceridemia and the development of fatty liver. Clin Chim Acta. 2001;314:85–91.
70. Gjesing AP, Andersen G, Burgdorf KS, Borch-Johnsen K, Jorgensen T, Hansen T, et al. Studies of the associations between functional beta2-adrenergic receptor variants and obesity, hypertension and type 2 diabetes in 7,808 white subjects. Diabetologia. 2007;50:563–8.
71. Jalba MS, Rhoads GG, Demissie K. Association of codon 16 and codon 27 beta 2-adrenergic receptor gene polymorphisms with obesity: a meta-analysis. Obesity (Silver Spring). 2008;16:2096–106.
72. Zhan Q, Li YY, Nie YQ, Zhou YJ, DU YL, Sha WH, et al. [Association of hepatic lipase gene promoter polymorphism -514C/T with nonalcoholic fatty liver disease]. Zhonghua Gan Zang Bing Za Zhi. 2008;16:375–8.
73. Cenarro A, Artieda M, Gonzalvo C, Merino-Ibarra E, Aristegui R, Ganan A, et al. Genetic variation in the hepatic lipase gene is associated with combined hyperlipidemia, plasma lipid concentrations, and lipid-lowering drug response. Am Heart J. 2005;150:1154–62.
74. Isaacs A, Sayed-Tabatabaei FA, Njajou OT, Witteman JC, Van Duijn CM. The -514 C->T hepatic lipase promoter region polymorphism and plasma lipids: a meta-analysis. J Clin Endocrinol Metab. 2004;89:3858–63.
75. Kraja AT, Vaidya D, Pankow JS, Goodarzi MO, Assimes TL, Kullo IJ, et al. A bivariate genome-wide approach to metabolic syndrome: STAMPEED consortium. Diabetes. 2011;60:1329–39.
76. Demirag MD, Onen HI, Karaoguz MY, Dogan I, Karakan T, Ekmekci A, et al. Apolipoprotein E gene polymorphism in nonalcoholic fatty liver disease. Dig Dis Sci. 2007;52:3399–403.
77. Lee DM, Lee SO, Mun BS, Ahn HS, Park HY, Lee HS, et al. [Relation of apolipoprotein E polymorphism to clinically diagnosed fatty liver disease]. Taehan Kan Hakhoe Chi. 2002;8:355–62.

78. Sazci A, Akpinar G, Aygun C, Ergul E, Senturk O, Hulagu S. Association of apolipoprotein E polymorphisms in patients with non-alcoholic steatohepatitis. Dig Dis Sci. 2008;53:3218–24.
79. Yang MH, Son HJ, Sung JD, Choi YH, Koh KC, Yoo BC, et al. The relationship between apolipoprotein E polymorphism, lipoprotein (a) and fatty liver disease. Hepatogastroenterology. 2005;52:1832–5.
80. Anthopoulos PG, Hamodrakas SJ, Bagos PG. Apolipoprotein E polymorphisms and type 2 diabetes: a meta-analysis of 30 studies including 5423 cases and 8197 controls. Mol Genet Metab. 2010;100:283–91.
81. Sookoian S, Castano G, Gemma C, Gianotti TF, Pirola CJ. Common genetic variations in CLOCK transcription factor are associated with nonalcoholic fatty liver disease. World J Gastroenterol. 2007;13:4242–8.
82. Garaulet M, Lee YC, Shen J, Parnell LD, Arnett DK, Tsai MY, et al. CLOCK genetic variation and metabolic syndrome risk: modulation by monounsaturated fatty acids. Am J Clin Nutr. 2009;90:1466–75.
83. Dongiovanni P, Valenti L, Rametta R, Daly AK, Nobili V, Mozzi E, et al. Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease. Gut. 2010;59:267–73.
84. McAteer JB, Prudente S, Bacci S, Lyon HN, Hirschhorn JN, Trischitta V, et al. The ENPP1 K121Q polymorphism is associated with type 2 diabetes in European populations: evidence from an updated meta-analysis in 42,042 subjects. Diabetes. 2008;57:1125–30.
85. Grarup N, Urhammer SA, Ek J, Albrechtsen A, Glumer C, Borch-Johnsen K, et al. Studies of the relationship between the ENPP1 K121Q polymorphism and type 2 diabetes, insulin resistance and obesity in 7,333 Danish white subjects. Diabetologia. 2006;49:2097–104.
86. Wang R, Zhou D, Xi B, Ge X, Zhu P, Wang B, et al. ENPP1/PC-1 Gene K121Q Polymorphism Is Associated with Obesity in European Adult Populations: Evidence from A Meta-Analysis Involving 24 324 Subjects. Biomed Environ Sci. 2011;24:200–6.
87. Morini E, Prudente S, Succurro E, Chandalia M, Zhang YY, Mammarella S, et al. IRS1 G972R polymorphism and type 2 diabetes: a paradigm for the difficult ascertainment of the contribution to disease susceptibility of ''low-frequency-low-risk'' variants. Diabetologia. 2009;52:1852–7.
88. Florez JC, Sjogren M, Burtt N, Orho-Melander M, Schayer S, Sun M, et al. Association testing in 9,000 people fails to confirm the association of the insulin receptor substrate-1 G972R polymorphism with type 2 diabetes. Diabetes. 2004;53:3313–8.
89. Tokushige K, Hashimoto E, Noto H, Yatsuji S, Taniai M, Torii N, et al. Influence of adiponectin gene polymorphisms in Japanese patients with non-alcoholic fatty liver disease. J Gastroenterol. 2009;44:976–82.
90. Wang ZL, Xia B, Shrestha U, Jiang L, Ma CW, Chen Q, et al. Correlation between adiponectin polymorphisms and non-alcoholic fatty liver disease with or without metabolic syndrome in Chinese population. J Endocrinol Invest. 2008;31:1086–91.
91. Musso G, Gambino R, De MF, Durazzo M, Pagano G, Cassader M. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: Possible pathogenetic role in NASH. Hepatology. 2008;47:1167–77.
92. Wong VW, Wong GL, Tsang SW, Hui AY, Chan AW, Choi PC, et al. Genetic polymorphisms of adiponectin and tumor necrosis factor-alpha and nonalcoholic fatty liver disease in Chinese people. J Gastroenterol Hepatol. 2008;23:914–21.
93. Perez-Martinez P, Lopez-Miranda J, Cruz-Teno C, gado-Lista J, Jimenez-Gomez Y, Fernandez JM, et al. Adiponectin gene variants are associated with insulin sensitivity in response to dietary fat consumption in Caucasian men. J Nutr. 2008;138:1609–14.
94. Gonzalez-Sanchez JL, Zabena CA, Martinez-Larrad MT, Fernandez-Perez C, Perez-Barba M, Laakso M, et al. An SNP in the adiponectin gene is associated with decreased serum adiponectin levels and risk for impaired glucose tolerance. Obes Res. 2005;13:807–12.
95. Tso AW, Sham PC, Wat NM, Xu A, Cheung BM, Rong R, et al. Polymorphisms of the gene encoding adiponectin and glycaemic outcome of Chinese subjects with impaired glucose tolerance: a 5-year follow-up study. Diabetologia. 2006;49:1806–15.
96. Yu Z, Han S, Cao X, Zhu C, Wang X, Guo X. Genetic Polymorphisms in Adipokine Genes and the Risk of Obesity: A Systematic Review and Meta-Analysis. Obesity (Silver Spring). 2011 Jun 9. doi:10.1038/oby.2011.148.
97. Dongiovanni P, Rametta R, Fracanzani AL, Benedan L, Borroni V, Maggioni P, et al. Lack of association between peroxisome proliferator-activated receptors alpha and gamma2 polymorphisms and progressive liver damage in patients with non-alcoholic fatty liver disease: a case control study. BMC Gastroenterol. 2010;10:102.
98. Chen S, Li Y, Li S, Yu C. A Val227Ala substitution in the peroxisome proliferator activated receptor alpha (PPAR alpha) gene associated with non-alcoholic fatty liver disease and decreased waist circumference and waist-to-hip ratio. J Gastroenterol Hepatol. 2008;23:1415–8.
99. Rey JW, Noetel A, Hardt A, Canbay A, Alakus H, Zur HA, et al. Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma2 in patients with fatty liver diseases. World J Gastroenterol. 2010;16:5830–7.
100. Huguenin GV, Rosa G. The Ala allele in the PPAR-gamma2 gene is associated with reduced risk of type 2 diabetes mellitus in Caucasians and improved insulin sensitivity in overweight subjects. Br J Nutr. 2010;104:488–97.
101. Gouda HN, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JP. The association between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: a HuGE review and meta-analysis. Am J Epidemiol. 2010;171:645–55.
102. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science. 2007;316:1341–5.
103. Tonjes A, Scholz M, Loeffler M, Stumvoll M. Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with Pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. Diabetes Care. 2006;29:2489–97.
104. Gonzalez Sanchez JL, Serrano RM, Fernandez PC, Laakso M, Martinez Larrad MT. Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma-2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population. Eur J Endocrinol. 2002;147:495–501.
105. Huang X, Zhao J, Zhao T. Effects of peroxisome proliferator activated receptor-gamma 2 gene Pro12Ala polymorphism on fasting blood lipids: a meta-analysis. Atherosclerosis. 2011;215:136–44.
106. Barroso I, Luan J, Sandhu MS, Franks PW, Crowley V, Schafer AJ, et al. Meta-analysis of the Gly482Ser variant in PPARGC1A in type 2 diabetes and related phenotypes. Diabetologia. 2006;49:501–5.
107. Musso G, Gambino R, Pacini G, Pagano G, Durazzo M, Cassader M. Transcription factor 7-like 2 polymorphism modulates glucose and lipid homeostasis, adipokine profile, and hepatocyte apoptosis in NASH. Hepatology. 2009;49:426–35.
108. Gonzalez-Sanchez JL, Martinez-Larrad MT, Zabena C, Perez-Barba M, Serrano-Rios M. Association of variants of the TCF7L2 gene with increases in the risk of type 2 diabetes and the proinsulin:insulin ratio in the Spanish population. Diabetologia. 2008;51:1993–7.

109. Hu C, Zhang R, Wang C, Wang J, Ma X, Hou X, et al. Variants from GIPR, TCF7L2, DGKB, MADD, CRY2, GLIS3, PROX1 SLC30A8 and IGF1 are associated with glucose metabolism in the Chinese. PLoS One. 2010;5:e15542.
110. Ingelsson E, Langenberg C, Hivert MF, Prokopenko I, Lyssenko V, Dupuis J, et al. Detailed physiologic characterization reveals diverse mechanisms for novel genetic Loci regulating glucose and insulin metabolism in humans. Diabetes. 2010;59:1266–75.
111. Liu PH, Chang YC, Jiang YD, Chen WJ, Chang TJ, Kuo SS, et al. Genetic variants of TCF7L2 are associated with insulin resistance and related metabolic phenotypes in Taiwanese adolescents and Caucasian young adults. J Clin Endocrinol Metab. 2009;94:3575–82.
112. Yang Z, Wen J, Tao X, Lu B, Du Y, Wang M, et al. Genetic variation in the GCKR gene is associated with nonalcoholic fatty liver disease in Chinese people. Mol Biol Rep. 2011;38:1145–50.
113. Nettleton JA, McKeown NM, Kanoni S, Lemaitre RN, Hivert MF, Ngwa J, et al. Interactions of dietary whole-grain intake with fasting glucose- and insulin-related genetic loci in individuals of European descent: a meta-analysis of 14 cohort studies. Diabetes Care. 2010;33:2684–91.
114. Onuma H, Tabara Y, Kawamoto R, Shimizu I, Kawamura R, Takata Y, et al. The GCKR rs780094 polymorphism is associated with susceptibility of type 2 diabetes, reduced fasting plasma glucose levels, increased triglycerides levels and lower HOMA-IR in Japanese population. J Hum Genet. 2010;55:600–4.
115. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010;42:105–16.
116. Saxena R, Hivert MF, Langenberg C, Tanaka T, Pankow JS, Vollenweider P, et al. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat Genet. 2010;42:142–8.
117. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007;316:1331–6.
118. Haupt A, Thamer C, Heni M, Tschritter O, Machann J, Schick F, et al. Impact of variation near MC4R on whole-body fat distribution, liver fat, and weight loss. Obesity (Silver Spring). 2009;17:1942–5.
119. Scherag A, Dina C, Hinney A, Vatin V, Scherag S, Vogel CI, et al. Two new Loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early-onset extreme obesity in French and german stu*dy gro*ups. PLoS Genet. 2010;6:e1000916.
120. Wang D, Ma J, Zhang S, Hinney A, Hebebrand J, Wang Y, et al. Association of the MC4R V103I polymorphism with obesity: a Chinese case-control study and meta-analysis in 55,195 individuals. Obesity (Silver Spring). 2010;18:573–9.
121. Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet. 2009;41:25–34.
122. Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, et al. Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat Genet. 2008;40:768–75.
123. Young EH, Wareham NJ, Farooqi S, Hinney A, Hebebrand J, Scherag A, et al. The V103I polymorphism of the MC4R gene and obesity: population based studies and meta-analysis of 29 563 individuals. Int J Obes (Lond). 2007;31:1437–41.
124. Geller F, Reichwald K, Dempfle A, Illig T, Vollmert C, Herpertz S, et al. Melanocortin-4 receptor gene variant I103 is negatively associated with obesity. Am J Hum Genet. 2004;74:572–81.
125. Oruc N, Ozutemiz O, Berdeli A, Ersoz G, Gunsar F, Karasu Z, et al. Common SPINK-1 mutations do not predispose to the development of non-alcoholic fatty liver disease. Ann Hepatol. 2009;8:116–9.
126. Lu H, Sun J, Sun L, Shu X, Xu Y, Xie D. Polymorphism of human leptin receptor gene is associated with type 2 diabetic patients complicated with non-alcoholic fatty liver disease in China. J Gastroenterol Hepatol. 2009;24:228–32.
127. De Luis DA, Gonzalez SM, Aller R, Izaola O, Conde R. Influence of Lys656Asn polymorphism of the leptin receptor gene on insulin resistance in nondiabetic obese patients. J Diabetes Complications. 2008;22:199–204.
128. Portoles O, Sorli JV, Frances F, Coltell O, Gonzalez JI, Saiz C, et al. Effect of genetic variation in the leptin gene promoter and the leptin receptor gene on obesity risk in a population-based case-control study in Spain. Eur J Epidemiol. 2006;21:605–12.
129. Heo M, Leibel RL, Fontaine KR, Boyer BB, Chung WK, Koulu M, et al. A meta-analytic investigation of linkage and association of common leptin receptor (LEPR) polymorphisms with body mass index and waist circumference. Int J Obes Relat Metab Disord. 2002;26:640–6.
130. Heo M, Leibel RL, Boyer BB, Chung WK, Koulu M, Karvonen MK, et al. Pooling analysis of genetic data: the association of leptin receptor (LEPR) polymorphisms with variables related to human adiposity. Genetics. 2001;159:1163–78.
131. Paracchini V, Pedotti P, Taioli E. Genetics of leptin and obesity: a HuGE review. Am J Epidemiol. 2005;162:101–14.
132. Aller R, De Luis DA, Izaola O, Gonzalez SM, Conde R, Alvarez GT, et al. G308A polymorphism of TNF-alpha gene is associated with insulin resistance and histological changes in non alcoholic fatty liver disease patients. Ann Hepatol. 2010;9:439–44.
133. Hu ZW, Luo HB, Xu YM, Guo JW, Deng XL, Tong YW, et al. Tumor necrosis factor-alpha gene promoter polymorphisms in Chinese patients with nonalcoholic fatty liver diseases. Acta Gastroenterol Belg. 2009;72:215–21.
134. Tokushige K, Takakura M, Tsuchiya-Matsushita N, Taniai M, Hashimoto E, Shiratori K. Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. J Hepatol. 2007;46:1104–10.
135. Valenti L, Fracanzani AL, Dongiovanni P, Santorelli G, Branchi A, Taioli E, et al. Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology. 2002;122:274–80.
136. Sookoian SC, Gonzalez C, Pirola CJ. Meta-analysis on the G-308A tumor necrosis factor alpha gene variant and phenotypes associated with the metabolic syndrome. Obes Res. 2005;13:2122–31.
137. Qi L, Van Dam RM, Meigs JB, Manson JE, Hunter D, Hu FB. Genetic variation in IL6 gene and type 2 diabetes: tagging-SNP haplotype analysis in large-scale case-control study and meta-analysis. Hum Mol Genet. 2006;15:1914–20.
138. Qi L, Zhang C, Van Dam RM, Hu FB. Interleukin-6 genetic variability and adiposity: associations in two prospective cohorts and systematic review in 26,944 individuals. J Clin Endocrinol Metab. 2007;92:3618–25.
139. Brun P, Castagliuolo I, Floreani AR, Buda A, Blasone L, Palu G, et al. Increased risk of NASH in patients carrying the C(-159)T polymorphism in the CD14 gene promoter region. Gut. 2006;55:1212.
140. Fernandez-Real JM, Broch M, Richart C, Vendrell J, Lopez-Bermejo A, Ricart W. CD14 monocyte receptor, involved in the inflammatory cascade, and insulin sensitivity. J Clin Endocrinol Metab. 2003;88:1780–4.
141. Al-Serri A, Anstee QM, Valenti L, Nobili V, Leathart JB, Dongiovanni P, et al. The sod2 c47t polymorphism influences nafld fibrosis severity: evidence from case-control and intra-familial

allele association studies. J Hepatol. 2011 Jul 11.[ahead of print].
142. Iwata R, Baur K, Stieger B, Mertens JC, Daly AK, Frei P, et al. A common polymorphism in the ABCB11 gene is associated with advanced fibrosis in hepatitis C but not in non-alcoholic fatty liver disease. Clin Sci (Lond). 2011;120:287–96.
143. Sookoian S, Castano G, Gianotti TF, Gemma C, Pirola CJ. Polymorphisms of MRP2 (ABCC2) are associated with susceptibility to nonalcoholic fatty liver disease. J Nutr Biochem. 2009;20:765–70.
144. Wang CW, Lin HY, Shin SJ, Yu ML, Lin ZY, Dai CY, et al. The PNPLA3 I148M polymorphism is associated with insulin resistance and nonalcoholic fatty liver disease in a normoglycaemic population. Liver Int. 2011;31:1326–31.
145. Nakayama K, Bayasgalan T, Yamanaka K, Kumada M, Gotoh T, Utsumi N, et al. Large scale replication analysis of loci associated with lipid concentrations in a Japanese population. J Med Genet. 2009;46:370–4.
146. Bille DS, Banasik K, Justesen JM, Sandholt CH, Sandbaek A, Lauritzen T, et al. Implications of Central Obesity-Related Variants in LYPLAL1, NRXN3 MSRA, and TFAP2B on Quantitative Metabolic Traits in Adult Danes. PLoS One. 2011;6: e20640.
147. Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. Nat Genet. 2010;42:949–60.
148. Hotta K, Nakamura M, Nakamura T, Matsuo T, Nakata Y, Kamohara S, et al. Polymorphisms in NRXN3, TFAP2B, MSRA, LYPLAL1 FTO and MC4R and their effect on visceral fat area in the Japanese population. J Hum Genet. 2010;55:738–42.
149. Lindgren CM, Heid IM, Randall JC, Lamina C, Steinthorsdottir V, Qi L, et al. Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. PLoS Genet. 2009;5:e1000508.
150. Dunn JS, Mlynarski WM, Pezzolesi MG, Borowiec M, Powers C, Krolewski AS, et al. Examination of PPP1R3B as a candidate gene for the type 2 diabetes and MODY loci on chromosome 8p23. Ann Hum Genet. 2006;70:587–93.
151. Luo X, Zhang Y, Ruan X, Jiang X, Zhu L, Wang X, et al. Fasting-induced protein phosphatase 1 regulatory subunit contributes to postprandial blood glucose homeostasis via regulation of hepatic glycogenesis. Diabetes. 2011;60:1435–45.
152. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 2010;466:707–13.