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Emerging role of genomic analysis in clinical evaluation of lean individuals with NAFLD

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Abstract

Whereas the rising prevalence of nonalcoholic fatty liver disease (NAFLD) is closely related with the global obesity epidemic, up to 10–20% of individuals with NAFLD are lean as defined by a body mass index of < 25 kg/m^2, or < 23 kg/m^2 in Asians. This entity designated as "lean NAFLD" is estimated to affect 8 to 10 million individuals in the United States alone. Here, we review the emerging data on the epidemiology, natural history and prognosis of lean NAFLD and put forward a diagnostic approach that combines detailed clinical phenotyping with genomic analysis. We propose two subtypes of lean NAFLD referred to as type 1: individuals with visceral adiposity and insulin resistance but normal BMI; and type 2: lean individuals with hepatic steatosis secondary to a known or unknown monogenic disease. We envision that incorporation of genomic analysis in the diagnostic algorithm of lean patients with NAFLD will elucidate the contribution of common genetic variants through the calculation of NAFLD polygenic risk score and also characterize the diverse array of rare monogenic diseases that can lead to triglyceride accumulation in the cytoplasm of hepatocytes. Collectively, the integration of a molecular diagnosis in the clinical evaluation of patients with lean NAFLD will provide an accurate diagnosis, with possible targeted therapies and may uncover novel molecular mechanisms with potential broader therapeutic implications.

Introduction

While the rising prevalence of nonalcoholic fatty liver disease (NAFLD) is closely associated with the global obesity epidemic, approximately 10–20% of individuals with

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AUTHOR CONTRIBUTIONS

All authors contributed to study concept and design, drafting of the manuscript, critical revision and approval of the final manuscript.

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Conflict of interests:

Potential conflict of interest for Rohit Loomba: Dr. Loomba serves as a consultant or advisory board member for Bird Rock Bio, Celgene, Enanta, GRI Bio, Madrigal, Metacrine, NGM, Sanofi, Arrowhead Research, Galmed, NGM, GNI, NovoNordisk, Merck, Siemens, Pfizer, Gilead. Glympsebio, In addition, his institution has received grant support from Allergan, BMS, BI, Daiichi-Sankyo Inc., Eli-Lilly, Galectin, Galmed, GE, Genfit, Intercept, Janssen Inc, Madrigal, Merck, NGM, Pfizer, Prometheus, Siemens, and Sirius. He is also co-founder of Liponexus Inc.

NAFLD have a body mass index (BMI) within the normal range (1–3). This sub-population is frequently described as “lean NAFLD”, and more precisely referred to as NAFLD among lean individuals.

NAFLD in patients who are lean, overweight or obese encompasses a diverse and heterogeneous set of entities. While most lean patients with NAFLD will share the same drivers as overweight and obese NAFLD individuals, a subset may be driven by rare genetic variants. Liver biopsy is considered the gold standard for diagnosis and prognostication of NAFLD. Liver biopsy assessment provides confirmation of pattern of injury, distribution and grade of steatosis, inflammation and hepatocyte ballooning, and stage of fibrosis. Furthermore, liver biopsy can also help in ruling out other causes of elevated serum aminotransferases and point towards a different pattern of liver injury that is not consistent with NAFLD (4). However, given the rising adoption of non-invasive biomarker-based risk assessment using clinical prediction rules (e.g.: FIB-4) and elastography-based tests (i.e., vibration controlled transient elastography or magnetic resonance elastography), liver biopsy utilization and access is dwindling in clinical practice outside of clinical trial enrollment. Hence, there remains a pressing need to further delineate the broad diagnosis of NAFLD at the molecular level, particularly among those with lean NAFLD, which will result in a better understanding of the medical entities to be considered in the differential diagnosis of lean NAFLD.

Here, we review the epidemiology, natural history, prognosis, pathogenesis and put forward a new diagnostic approach to this population that incorporates clinical evaluation and genomic analysis. The purpose of this review is to fill the gap in knowledge regarding when to entertain a broad differential diagnosis in the evaluation of a lean individual who presents with suspected NAFLD. We will propose a diagnostic algorithm that may be useful in a clinical setting along with a justification for sending out genetic testing to uncover masquerading specific genetic disorders that may present as NAFLD. Pairing physical examination, laboratory, imaging, and biopsy findings with genomic information will provide a molecular diagnosis with insight into disease pathogenesis, ultimately guiding therapeutic options, preventative medicine interventions, and appropriate family counseling (5, 6).

Epidemiology, Natural History and Prognosis of Lean NAFLD

NAFLD in lean patients comprises a significant minority of NAFLD cases, ranging from 10–20% of all NAFLD cases worldwide (7). The highest prevalence of lean NAFLD is described in Asia, where a lower BMI threshold for overweight, > 23 kg/m^2, is used (2). Epidemiologic studies describe lean patients with NAFLD as more often male, older, and with larger waist circumference than lean patients without NAFLD but have lesser degrees of metabolic abnormalities compared to overweight/obese NAFLD (8). Importantly, even up to 8% of lean adolescents may also have NAFLD (9), although this remains significantly lower than prevalence among overweight and obese adolescents (10).

The impact of lean NAFLD on long-term disease severity and prognosis is incompletely understood, however, emerging data suggests that disease progression is slower than in those with obesity and NAFLD (11, 12). Hagstrom and colleagues performed a cohort study of

646 patients with biopsy proven NAFLD of whom 123 had BMI < 25 kg/m^2 and found no increase in overall mortality, but a possible increased risk of severe liver disease among those with lean NAFLD when compared to a reference population of NAFLD with BMI 25–30 kg/m^2 (13). Another retrospective study found that lean patients with NAFLD had less non-alcoholic steatohepatitis (NASH) and less significant fibrosis seen on biopsy than patients with overweight/obese NAFLD (12). In an Asian cohort that compared non-obese (including overweight) to obese NAFLD subjects, non-obese patients with NAFLD had less severe liver disease at baseline and a better prognosis, albeit with a limited number of events (11). Finally, a retrospective study of biopsy proven NAFLD patients found lean patients to have less steatosis but more hepatocyte ballooning, lobular inflammation, and cirrhosis than overweight patients with NAFLD (14). Lifestyle modification remains the backbone of treatment for NAFLD in lean patients with visceral adiposity and insulin resistance as supported by a small randomized controlled trial of lifestyle intervention demonstrating that a 3–5% weight reduction was beneficial for non-obese NAFLD (15). However, lean patients with NAFLD caused by monogenic disorders may not respond to lifestyle modifications alone and targeted therapies may be needed.

With regards to cardiovascular events, lean NAFLD patients may have a similar risk to overweight and obese NAFLD patients (11–13). Hepatic steatosis remains associated with a greater risk of major adverse cardiovascular events even after adjustment for obesity (16) and in patients with biopsy proven NAFLD, BMI was not associated with the development of cardiovascular disease (17). Traditional cardiovascular risk factors and the presence of advanced fibrosis are more strongly associated with cardiovascular disease than BMI in patients with NAFLD (18). The diverse underlying etiologies that contribute to the development of lean NAFLD, which are not systematically evaluated in the published literature, likely contribute to the contradictory associations with NAFLD severity. Furthermore, spectrum effects may lead to bias when a cross-sectional study includes patients with advanced liver disease who may have progressive sarcopenia and decreasing BMI. Therefore, there is a significant need to systematically evaluate natural history and risk factors of progression of NAFLD in lean individuals.

Diagnostic Approach

Lean individuals with NAFLD likely represent a heterogenous group of molecular diagnoses. The initial approach is identical to the diagnosis of any patient with NAFLD and should consist in excluding secondary causes of hepatic steatosis including drugs, excessive alcohol intake and other known causes of chronic liver disease. In cases of fatty liver in lean individuals, an alcohol use history may be coupled with sensitive biomarkers of alcohol use including urine ethyl glucuronide (detection within 3–5 days) and blood phosphatidylethanol (detection within 1–2 weeks) to exclude alcohol overuse or abuse (19). We propose delineation of two broad subtypes of lean NAFLD here onward referred to as Type 1: individuals with visceral adiposity and insulin resistance but normal BMI, and Type 2**:** individuals in which hepatic steatosis results from a monogenic disorder, and therefore with rare genetic variants driving disease (Figure 1). The majority of lean NAFLD patients are Type 1, lean by BMI thresholds but obese by waist circumference or other measures of body composition and whom present with visceral adiposity and insulin resistance (20). The

pathogenesis of NAFLD in this subtype likely parallels overweight and obese individuals with NAFLD and is driven by excessive caloric intake, particularly simple carbohydrates and sedentary lifestyle (21) leading to hepatic steatosis and lipotoxicity. Evaluation for insulin resistance should include physical examination to evaluate for the presence of abdominal obesity (i.e. measurement of waist circumference) and acanthosis nigricans; and laboratory tests, namely fasting glucose and insulin levels, HOMA-IR calculation, and lipid panel (total cholesterol, LDL, HDL and triglyceride levels).

Common Genetic Variants and NAFLD Risk

Importantly, the cumulative impact of common genetic variants, typically called polymorphisms, associated with NAFLD may contribute to the manifestation of disease despite a lower BMI (22). These lean patients with NAFLD likely have a higher prevalence of common variants associated with NAFLD prevalence and severity (23). The most well characterized common variant associated with NAFLD is the nonsynonymous variant p.I148M in the *PNPLA3* gene, which has increased prevalence in Hispanics (24) and is associated with an increased risk of hepatic steatosis (24), NASH (25, 26), cirrhosis (27) and hepatocellular carcinoma (28). Stender and colleagues demonstrated that adiposity, assessed by BMI, amplifies the effect of this *PNPLA3* polymorphism on fatty liver disease and its progression to cirrhosis; however, this finding was in a population of European ancestry (29). Mechanistic studies suggest that this *PNPLA3* variant increases liver fat when challenged with high sucrose diets (30) and in East Asian and South Asian populations increased visceral fat may develop despite a normal BMI (31). In addition, the *TM6SF2* variant p.E167K increases susceptibility to NAFLD (32) but may protect against cardiovascular disease by impeding normal VLDL assembly and decreasing circulating lipids (33). The interaction between the *TM6SF2* risk variant and adiposity on liver fat was present but less significant (29) than observed with *PNPLA3*. In animal models the *TM6SF2* risk variant can cause fatty liver on a normal diet (34) but may be exacerbated by high fat and fructose diets (35). Phenome-wide association studies demonstrate an association between the *TM6SF2* risk variant and lower peripheral fat and BMI (36) suggesting the risk variant may play an important role in lean individuals with NAFLD. Polymorphisms in *MBOAT7* (37) and *GCKR* (38) have also been associated with NAFLD, albeit with more modest effects. *MBOAT7* variants associated with liver disease are not clearly associated with obesity or BMI on phenome-wide association studies (36). The effect of *GCKR* risk variants on liver fat are amplified by obesity and expression is increased by glucose and insulin (29). On the other hand, variants in *HSD17B13* (39, 40) and *MARC1* (41, 42) have demonstrated protective effects against liver disease severity, however, evaluation for interaction with adiposity requires further study. Recently published reviews further detail the impact of genetic variants in NAFLD (43–45). Taken together the contribution of multiple genetic traits can be incorporated into a weighted polygenic risk score that incorporates the cumulative impact of multiple common variants. Risk scores incorporating several loci have demonstrated associations with hepatic triglyceride content, aminotransferases, and hepatocellular carcinoma risk (46, 47). Scores incorporating a greater number of common genetic variants may identify high risk individuals whose polygenic risk approaches the risk of rare monogenic variants (48). Recently a polygenic risk score for

cirrhosis incorporating twelve independent genetic variants was derived and validated (27). Future efforts should evaluate if lean patients with NAFLD have high polygenic risk score.

Rare Genetic Variants and Type 2 Lean NAFLD

A subset of lean patients with NAFLD and without visceral adiposity may have disease driven by rare genetic variants, which in certain cases may be amenable to targeted treatments. The identification of the underlying molecular mechanism(s) can lead to a precise diagnosis, with implications in the delivery of personalized therapy, preventative medicine intervention(s), and appropriate family counseling (Figure 1). Hence, in these lean patients without visceral adiposity, genetic evaluation should be considered (Figure 1) (5, 6). Additionally, the identification of an atypical phenotype or systemic symptoms in a lean patient with NAFLD (Table 1) should prompt further evaluation. A detailed review of history, physical exam, laboratory, and imaging findings paired with genomic analysis can uncover a variety of disorders presenting with hepatic steatosis and commonly referred to a hepatologist for “lean NAFLD” (Table 1, Figure 1). This review aims to alert hepatologists to consider broadening the differential diagnosis in these cases and perform genetic evaluation in patients with Type 2 “lean NAFLD”.

Lipoprotein and Lipid Disorders—The liver is a major player in the processing of lipids, which includes its utilization in ATP generation, the secretion as lipoproteins, or the storage of excess for future use (49). Imbalance in any of these processes essential for lipid metabolism homeostasis can lead to their accumulation in hepatocytes, manifesting as hepatic steatosis.

Hypobetalipoproteinemia (HBL) is a result of defective apolipoprotein B lipid transport due to heterozygous or homozygous loss-of-function mutations in *APOB* (50). While the recessive form of HBL is rare and presents with a more severe phenotype featuring fat malabsorption and liposoluble vitamin deficiencies, it is estimated that 1 in 3,000 individuals worldwide harbor a heterozygous loss-of-function mutation in *APOB* which can present with low circulating lipid levels and hepatic steatosis (Table 1) (5, 51, 52). In contrast to patients with metabolic syndrome, these patients typically have fasting lipid profiles demonstrating low circulating triglyceride, apoB, and low-density lipoprotein (LDL) cholesterol levels. While longitudinal studies following these patients after initial diagnosis are limited, hepatic steatosis in some patients with lipoprotein disorders has progressed to advanced fibrosis and hepatocellular carcinoma (53, 54), underscoring the importance of prompt recognition and diagnosis and long-term monitoring for these patients. A recent analysis of the UK biobank revealed that 0.8% of participants with hepatic steatosis on MRI had a loss-of-function variant in either *APOB* or *MTTP*, which plays a key role in the export of lipids from the liver (55).

Monoallelic mutations in *ABHD5*, which encodes abhydrolase domain-containing 5/ lysophosphatidic acid, resulting in ABHD5 insufficiency have been implicated as the cause of a rare heritable form of NAFLD in individuals older than 40 years of age due to an impairment in neutral lipid metabolism (56).

Lysosomal acid lipase deficiency (LAL-D) is an autosomal recessive disorder that results from loss of function mutations in *LIPA*, which encodes lipase A, that impair the hydrolysis of cholesteryl esters and triglycerides leading to its accumulation in hepatocytes, macrophages, and the spleen. For this reason, patients with *LIPA* deficiency can develop hepatic steatosis and dysfunction progressing to cirrhosis associated with dyslipidemia. The disease exists as a spectrum. The extreme phenotype is Wolman disease, which presents with liver failure in infancy. Patients with a lower level of activity are considered to have cholesteryl ester storage disease which can present with a combination of hepatic steatosis, dyslipidemia (typically characterized by extremely low HDL cholesterol) and a predisposition for coronary artery disease. A recent meta-analysis of 9 population-based studies identified the prevalence at 1 per 177,452 (57). The prevalence was highest in those of European and Latino ancestry and lower in East Asian, South Asian, Finnish and Ashkenazi Jewish ancestry. Diagnostic testing involves enzyme activity testing, in addition to genetic testing. Importantly, there is a targeted treatment available with sebelipase alpha.

II. Lipodystrophy Syndromes—Lipodystrophy syndromes, with an estimated prevalence of 1.3 – 4.7 cases per million, encompass a spectrum of diseases characterized by selective loss of peripheral adipose tissue; several forms are associated with ectopic fat deposition in visceral tissues such as the liver (58, 59). The most common hepatic manifestation of lipodystrophy is NAFLD; in a study of 50 patients with different forms of lipodystrophy who underwent liver biopsy, 82% scored as either borderline or definite steatohepatitis (60). Lipodystrophies can often be differentiated from traditional NAFLD by decreased leptin levels, since leptin is typically secreted from adipocytes (60). Dual energy X-ray absorptiometry is a burgeoning tool for objectively assessing adipose tissue distribution to aid in the diagnosis of lipodystrophy (61). Familial partial lipodystrophy (FPLD) is a genetic disorder characterized by loss of limb adiposity, insulin resistance, hepatic steatosis, hypertriglyceridemia, and an increased risk of cardiovascular disorders (62). Six subtypes of FPLD (types 1–6) have been described thus far; FPLD types 2 and 3 are the two most common forms reported in over 500 patients and 20 families, respectively (63). Leptin replacement therapy has shown promise for patients with lipodystrophy in improving insulin resistance and triglyceride levels (5, 62). In follow-up liver biopsy studies of patients with lipodystrophy and NASH, leptin therapy was associated with improvement in both hepatic steatosis as well as NASH related activity and stabilization of fibrosis (60).

III. Mitochondrial Disorders—As a primary site of glycolysis and beta-oxidation, the hepatocyte is a mitochondria-rich cell. Mutations in either mitochondrial DNA or genomic DNA encoding mitochondrial proteins can result in the breakdown of fatty acid oxidation or accumulation of reactive oxygen species. Mitochondrial disorders are heterogeneous entities that affect approximately 1 in 5,000 individuals worldwide (64) and often feature neuromuscular, psychiatric, cardiac and/or hepatic symptoms due to higher energy requirements in the brain, muscle and liver (5, 65, 66). While liver findings may not be the most severe in a patient’s phenotypic presentation, in some cases they are an important early indication of disease. For instance, a non-obese adult patient evaluated in liver clinic for NAFLD of unknown cause was found to harbor a homozygous missense mutation in *NDUFB3*, which encodes an accessory subunit of the complex I in the electron

transport chain of the mitochondria. Interestingly, he also had short stature, dysmorphic facial features, and oculomotor and optic nerve dysfunction (5).

Citrullinemia type 2 (CTLN2) is an autosomal recessive disorder due to mutations in *SLC25A13*, which encodes mitochondrial aspartate glutamate carrier 2. This disease is characterized by the sudden onset of neuropsychiatric symptoms such as coma due to hyperammonemia, which in some cases may require emergent liver transplant; hepatic steatosis is also seen in patients with CTLN2. In a retrospective study of 19 adult patients diagnosed with CTLN2, 89% had fatty liver at time of admission, and 26% had prior knowledge of fatty liver findings before neuropsychiatric symptoms onset (67). In these patients with CTLN2 and fatty liver, none were classified as obese based on either BMI or waist circumference measurement. Early guidance of these patients to avoid a carbohydrate-heavy diet can mitigate strains on NADH/NAD+ ratios in the mitochondria and avoid the progression to hyperammonemia and its complications (68).

NAFLD, Endocrine Disorders and Other Medical Entities—Endocrine disorders including panhypopituitarism can lead to NAFLD and may be at an increased risk for rapidly progressive disease and possibly amenable to hormone supplementation (69). Symptoms may be gradual in onset and non-specific, including fatigue, muscle weakness, reproductive changes and change in body composition if initially presenting in adulthood. Furthermore, isolated growth hormone deficiency may contribute to hepatic steatosis and supplementation may mitigate liver injury (70). Turner syndrome, the most common sex chromosome disorder, has been associated with NAFLD commonly in overweight, non-obese individuals and can be considered when the presentation is associated short stature and delayed puberty (71). Lastly, thyroid dysfunction may be associated with an increased risk for NAFLD (72, 73) and should be considered in lean patients with NAFLD.

Weber-Christian disease is a rare disorder of unknown etiology that may cause “lean NAFLD” by higher mobilization of liver fat (74). Patients may present with panniculitis manifested as subcutaneous nodules, fever, arthralgia and may not have traditional features of obesity and metabolic syndrome. Importantly, immunosuppression may treat the underlying symptoms.

Wilson disease affects 1 in 10,000–30,0000 people worldwide and is caused by recessive loss-of-function mutations in *ATP7B*, which encodes ATPase copper transporting beta, resulting in impaired copper transport and abnormal copper accumulation (75). This can lead to both acute liver failure as well as chronic liver disease leading to cirrhosis, and neuropsychiatric perturbations. Hepatic steatosis may be one of the first presentations of Wilson disease and may precede neurologic symptoms for years (76). Notably, in early stages of disease, liver biopsy may show steatosis with no clear evidence of copper overload, leading to the misdiagnosis of NAFLD in some cases (77).

Future Directions

The systematic evaluation of lean patients with NAFLD will ensure appropriate classification and treatment, particularly for those with Type 2 disease, driven by rare genetic variants. Furthermore, there have been several examples of how the investigation of

a rare presentation of disease has led to broader biomedical discoveries with therapeutic implications on common diseases. A classic illustration of this approach comes from the work from Brown and Goldstein, who identified that certain rare cases of familial hypercholesterolemia were a result of mutations in the LDL receptor. This finding has significantly contributed to our current understanding of cholesterol regulation and ushered in a new era of statin therapy, now the mainstay treatment of hyperlipidemia and coronary artery disease in the general population (78, 79). Similarly, deep phenotyping paired with genomic analysis of patients with "lean NAFLD" may uncover additional insight(s) into the pathogenesis of NAFLD with potential identification of novel therapeutic targets.

Large longitudinal population-based studies of lean patients with NAFLD paired with genotype information will be required to uncover the natural history of disease accounting for their heterogeneity in molecular diagnoses. Heterogeneity and the lack of molecular classification of disease may also contribute to lower efficacy of many pharmacologic interventions. Thus far, NAFLD clinical research has predominantly relied on inclusion criteria based solely on phenotypic features. Incorporating molecular diagnoses based on genetic testing results will improve the design of clinical trials overall, assisting in the proper selection and enrollment of patients so that the drug tested is in alignment with the patient's disease molecular pathogenesis (6). Newer therapies for treatment of NASH based upon genetic make-up are emerging. Antisense oligonucleotide approaches as well as small interfering RNA-based approaches to silence PNPLA3 are currently being evaluated in patients who are homozygous for the *PNPLA3* risk allele. It is also plausible that the knowledge of polygenetic risk score may help enrich for patients who are rapid progressors, and inclusion of such patients may reveal the efficiency of certain drugs, which can be obscured due to molecular heterogeneity of experimental group. Other possibilities exist where genotype may be linked to high or low likelihood of treatment response to a particular mechanism of action. In fact, emerging evidence supports that *PNPLA3* genotype may modulate response to existing treatments including omega-3 fatty acids (80, 81) and statins (82).

In conclusion, a clearer appreciation of the molecular subsets of NAFLD will illuminate which aspects of fatty liver disease onset and progression are shared and which differ, ultimately advancing our knowledge and the care of all patients along the hepatic steatosis spectrum. A systematic approach to the diagnostic evaluation of this population will improve our understanding of the wide spectrum of "lean NAFLD" and may provide a blueprint for how to incorporate clinical phenotyping and molecular diagnostics to define distinct NAFLD molecular subtypes.

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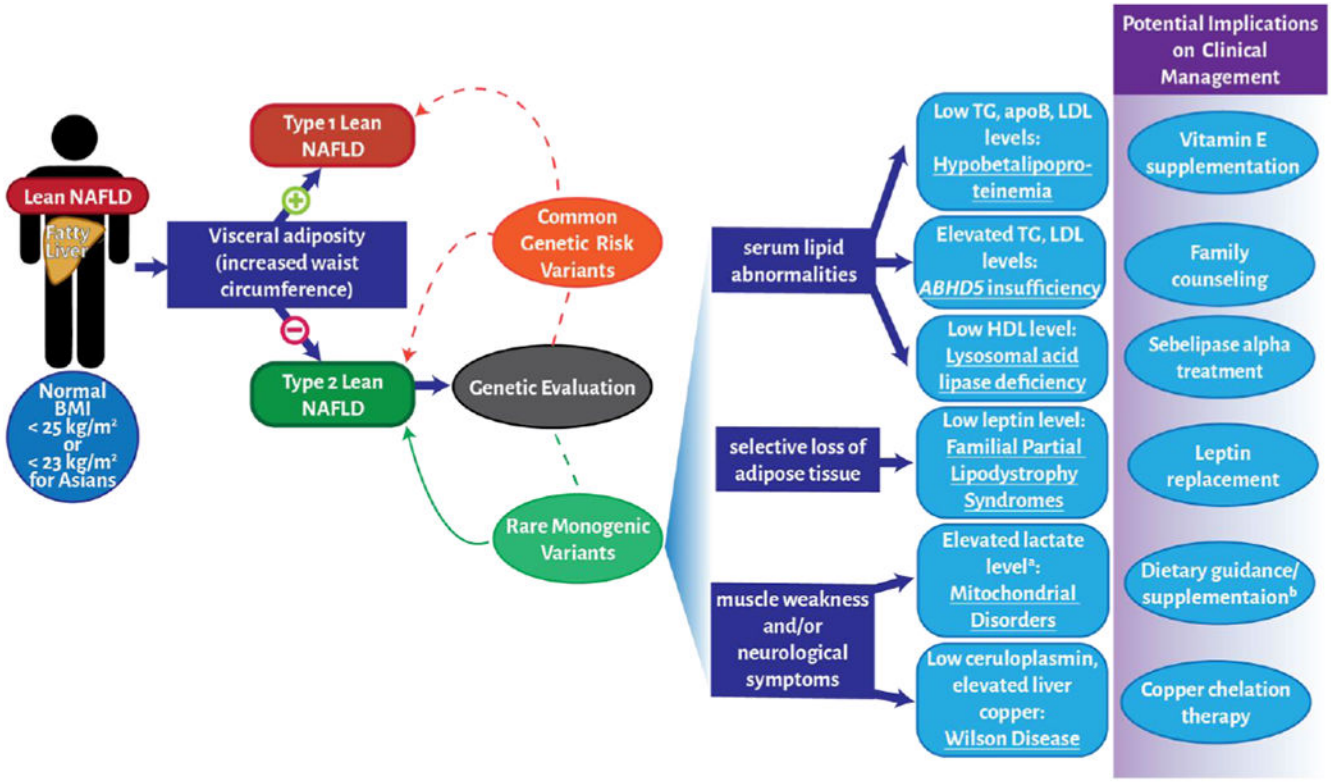

Figure 1:

Proposed diagnostic approach for uncovering underlying monogenic disorders in lean patients with NAFLD.

[a]Patients with mitochondrial disorders may present with elevations in lactic acid levels upon minor physiological stressors (e.g., minor surgery, fever/infection). [b]Dependent on the exact mitochondrial disorder, dietary guidance/supplementation may include avoidance of carbohydrate-rich foods or supplementation of different antioxidants, co-factors (e.g., arginine, CoQ10, L-carnitine).

Table 1.

Clinical features to be considered in lean patients presenting with presumed NAFLD that should trigger consideration of an underlying rare disorder.

History of…	Exam Findings of…	Laboratory/Imaging Findings of…	Consider …
Mild steatorrhea	Splenomegaly	Low circulating LDL-cholesterol and TG levels	*Lipoprotein and Lipid Disorders (i.e., ABHD5 insufficiency, hypobetalipoproteinemia, lysosomal acid lipase deficiency)*
Coronary artery disease		Low circulating apoB levels	
Insulin resistance	Areas of subcutaneous fat loss	Elevated fasting glucose level and Hgb A1c	*Lipodystrophy Syndromes*
Polycystic ovarian syndrome	Acanthosis nigricans	Elevated LH/FSH ratio	
Hypertriglyceridemia	Hypertension	Elevated triglycerides	
Pancreatitis	Hepatomegaly		
Preeclampsia			
Muscle weakness/fatigue	Abnormal MSK exam (e.g. reduced strength/ tone)	Elevated serum lactate	*Mitochondrial Disorders*
Lactic acidosis	Neurological deficits	Elevated serum ammonia levels	
Cardiomyopathy/heart block of unknown etiology	Dysmorphic facial features	Echocardiogram and EKG abnormalities	
Neuropsychiatric abnormalities	Short stature		
Seizure/epilepsy	Ophthalmoplegia/ptosis		
GI motility-related symptoms	Hearing loss		
Muscle weakness/fatigue	Dry skin	Low GH	*Endocrine Disorders (i.e., growth hormone deficiency)*
Menstrual irregularity	Brittle hair	Abnormal TSH	
Heat/cold intolerance			
Mood disorders			
Recurrent fever Malaise	Tender subcutaneous nodules		*Weber-Christian Disease*
Neuropsychiatric abnormalities	Kayser-Fleischer rings	Low ceruloplasmin level	*Wilson Disease*
Menstrual irregularity		Elevated liver copper level	

Note: While family history can increase clinical suspicion for genetic disease, its absence is not sensitive for lack of genetic disease as illustrated in Hakim et.al, 2019.

MSK, musculoskeletal; LH, luteinizing hormone; FSH, follicle stimulating hormone; EKG, electrocardiogram