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Haptoglobin 2 allele is associated with histological response to vitamin E in subjects with nonalcoholic steatohepatitis

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Conflicts of Interest:

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Abstract

Background: Haptoglobin (Hp) genotype has been linked to oxidative stress and response to vitamin E (VitE) in patients with dyslipidemia. Its effect on histological response to VitE in nonalcoholic steatohepatitis (NASH) is unknown.

Goals: Our objective was to determine if Hp genotype associates with response to VitE in patients with NASH.

Study: A post hoc analysis of 228 patients receiving VitE or placebo in two clinical trials was performed. Regression analysis was used to assess the effect of VitE versus placebo, by Hp genotype (1–1, 2–1, or 2–2), on histologic features and laboratory markers of liver disease, comparing baseline to end of treatment values. An interaction term was included in the regression models to assess differential treatment effect across Hp genotype.

Results: Hp 2–2 patients treated with VitE versus placebo showed significant histologic improvement (51% versus 20%, OR=4·2, p=0·006), resolution of steatohepatitis (44% versus 12%, OR=6.2, p=0·009), decrease in NAFLD Activity Score (NAS) (−2·2 versus −0·6, p=0·001), and decrease in liver enzymes alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ-glutamyl transpeptidase. Hp 2–1 patients on VitE versus placebo showed improved resolution of steatohepatitis, NAS and liver enzymes. Hp 1–1 patients showed no significant improvement in histology or liver enzymes. VitE had no effect on fibrosis stage in any group. Regression analysis showed incremental benefit of having Hp 2–2 or 2–1 versus 1–1 for all liver enzymes.

Conclusion: Hp 2 allele is associated with greater histological and biological improvement in NASH with VitE treatment compared to the Hp 1 allele.

Keywords

Nonalcoholic steatohepatitis; nonalcoholic fatty liver disease; vitamin E; haptoglobin genotype; oxidative stress

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease globally.[1] Within the spectrum of NAFLD, the subtype nonalcoholic steatohepatitis (NASH) is characterized by hepatocyte inflammation and ballooning that can progress to cirrhosis and liver cancer. The burden of liver disease attributable to NASH, and the lack of approved therapeutics underscore the importance of continued efforts towards therapeutic development. Oxidative stress is known to play a key role in the pathogenesis of NASH.[2,3] The antioxidant Vitamin E (VitE; RRR-α-tocopherol) has been shown to reduce steatosis, inflammation and ballooning, the key features of steatohepatitis.[4–6] In two separate placebo-control trials of VitE, the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial, and the Treatment of Nonalcoholic Fatty Liver Disease In Children (TONIC) trial, VitE was significantly superior to placebo in resolving steatohepatitis.[4,5] Both trials were conducted by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN).

Among the many agents that have been tested for NASH or are currently in therapeutic development, VitE has had the most substantial effect on disease activity.[7] Although the impact of VitE and other agents on clinically meaningful outcomes and progression to cirrhosis are yet to be demonstrated, current recommendations for therapeutics have focused on decreasing disease activity. VitE is recommended by both European and North American practice guidelines for the treatment of NASH.[1,8] Some studies have raised concerns about the long-term safety of VitE,[9,10] while other studies have challenged these concerns.[11,12] Identifying the subset of patients who respond optimally to VitE would serve a major step towards harnessing the utility of this inexpensive and widely available agent.

Haptoglobin (Hp) is an anti-oxidant protein produced by the liver that scavenges hemoglobin released during red blood cell turnover, intra- and extra-vascular hemolysis, thus preventing hemoglobin-mediated oxidative injury.[13] Two principal alleles of Hp (Hp 1 and Hp 2) have been identified and three distinct genotypes (Hp 1–1, Hp 2–1 and Hp 2–2) exist.[14,15] Hp 2–2 and 2–1 exhibit inferior antioxidant activity compared to Hp 1–1.[16–18] Diabetic individuals with Hp 2–2 have a 5-fold increased risk for cardiovascular disease compared to those with Hp 1–1.[19] Patients with cardiovascular disease and Hp 2–2 genotype treated with VitE demonstrate a significant decrease in atherogenic risk factors and cardiovascular outcomes.[19]

No studies have investigated the association between Hp genotype and pharmacologic response to VitE in patients with NASH. We performed a post hoc analysis of data from the PIVENS and TONIC trials to test the hypothesis that the presence of Hp 2 allele was associated with improved histological and biochemical outcomes following treatment with VitE. Our primary aim was to assess the relationship between Hp genotype, VitE supplementation, and improvement in the histological and biochemical features of NASH in patients enrolled in the PIVENS and TONIC trials.

MATERIALS AND METHODS

Clinical trials examined and study design

The PIVENS trial (NCT00063622)[4,5] was a prospective randomized controlled trial (RCT) of pioglitazone or VitE versus placebo in nondiabetic adult subjects with biopsy-proven NASH with a NAFLD activity score (NAS) of ≥4, and no cirrhosis as determined by a site pathologist. All liver biopsies were subsequently evaluated in a blinded manner by the central pathology committee of the NASH CRN.[4] The TONIC trial (NCT00063635) was another RCT that evaluated metformin or VitE versus placebo in nondiabetic children with NAFLD.[5] The entry criteria were similar to that of PIVENS; however, subjects with any histological pattern of NAFLD were included in TONIC.[4,5] Both the PIVENS and TONIC trials used a total daily dose of 800 IU VitE for 96 weeks.[4,5]

The current analysis included patients randomized to VitE or placebo in the PIVENS and TONIC trials who had both baseline and end of treatment (EOT) liver biopsy, as well as whole blood sample for DNA extraction. The analysis was approved by the Ancillary Studies committee of the NIDDK NASH CRN and all subjects provided consent for such analyses in their original DNA consent forms and thus did not require a separate IRB

approval. The analysis was performed collaboratively at the authors' institutions and the Data Coordinating Center of the NASH CRN. The manuscript was written entirely by the investigators who take full responsibility for its content and conclusions.

Haptoglobin genotyping

Hp genotype was determined using conventional polymerase chain reaction (PCR) as previously described the Invitrogen PCR kit.[20] Genomic DNA of patients in the PIVENS and TONIC trials were extracted from whole blood by the NASH CRN and stored at −80ºC. Primers A (5′-GAGGGGAGCTTGCCTTTCCATTG-3′) and B (5′-GAGATTTTTGAGCCCTGGCTGGT-3′) were used to amplify a 1757-bp sequence specific to Hp 1 and a 3481-bp sequence specific to Hp 2. Primers C (5′-CCTGCCTCGTATTAAACTGCACCAT-3′) and D (5′-CCGAGTGCTCCACATAGCCATGT-3′) were used to amplify a 349-bp sequence specific to Hp 2. As previously described, due to primer design, genotype determination was not compromised by sequence variations in haptoglobin allele subtypes.[20] Oligonucleotide primers were synthesized by Invitrogen.

PCR reactions were performed using Invitrogen PCR kit. Each reaction consisted of 1X PCR buffer, 200 μM of dNTP, 1·5 mM of $MgCl_2$, 0·2μM each of primers A and B or C and D, 1 Unit of Platinum Taq polymerase (Invitrogen), 80 to 110 ng of genomic DNA, and water to make up to 25μl. The PCR temperature profile consisted of denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 3 min, followed by a final extension at 72°C for 10 min. The thermocycler used was C1000 Touch Thermal Cycler (Bio-rad).

The PCR products for primers A/B and primers C/D were combined for each DNA sample and separated on 1% agarose gel with ethidium bromide in 1X Tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer. After resolution, bands were viewed and images captured under ultraviolet light. Hp 1–1 was identified by a sole 1757-bp band; Hp 2–2 by two bands at 3481-bp and 349-bp; and Hp 2–1 by bands at 1757-bp and 349-bp (and at times a faint band at 3481-bp).

Assessment of histological features

The assessment of liver histology in the PIVENS and TONIC trials has been previously described.[4,5] In both trials, liver histology was evaluated and scored centrally by the pathology committee of the NIDDK NASH CRN using the NASH CRN scoring system.[21] The NAFLD Activity Score (NAS (0–8)) is computed by summing Steatosis grade (0–3), Ballooning (0–2), and Lobular inflammation (0–3) scores. For regression analyses of fibrosis, sub-stages 1a, 1b and 1c were combined and classified as stage 1. Histological improvement at EOT from baseline was defined by a decrease in the NAFLD activity score (NAS) of 2 or more points without worsening of fibrosis. Resolution of NASH was defined as a change from "borderline or suspicious" or "definite NASH" to "NAFLD, not NASH" on EOT biopsy, excluding patients with "NAFLD, not NASH" on baseline biopsy.

Demographic, anthropometric, and laboratory biomarker data assessment

Demographic data were obtained from self-reported face-to-face surveys at each clinic. At each clinic visit, whole blood was obtained via venipuncture after an overnight fast and analyzed for biochemical data, including alkaline aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT). Baseline and EOT values were used to compute changes in biochemical measures.

Statistical analysis

The Hp genotype (1–1, 2–1, 2–2) distribution was determined for the total study population and compared to previously published distribution in Western populations using Fisher's exact test.[14,19,22] Continuous characteristics were expressed as mean ± standard deviation (SD) and as percentage for categorical variables. Continuous variables were compared across groups using ANOVA, and binary characteristics were compared using Fisher's exact test. For each Hp genotype, regression analyses were used to determine the treatment effect. The odds ratio (OR) and p-values (*p*) for the binary outcomes of histological improvement and NASH resolution were computed using logistic regression of the outcome in relation to the treatment group and for continuous outcomes, adjusted mean changes (adjM) over follow-up were calculated using ANCOVA, adjusting for the baseline value of the outcome; all models were adjusted for age group using an indicator of adult versus pediatric. To test this study's primary hypothesis that the treatment effect differed across genotypes, an interaction term (treatment and genotype) was included in the models assuming the genotypic model of association[23]. Tests of genetic association were performed for each individual genotype to determine the underlying genetic model of association with disease; if the Hp categories appear ordered, then the additive model was more powerful than genotypic. The additive model was analyzed by including Hp genotype in the regression model as an ordinal variable, where Hp 1–1=0, Hp 2–1=1 and Hp 2–2=2; if the model assumption of a linear relationship across the groups was met, then the interaction p-value for trend (P) was calculated. If the assumption of a linear relationship across the group was not met or the numbers were too small to calculate such assumption, then the interaction is denoted as not applicable (n/a) in the reported results. In sensitivity tests, all regression models were analyzed separately for PIVENS and TONIC without adjustment for age group. Since there was a higher proportion of Hispanics in the Hp 1–1 genotype compared to Hp 2–2 and 2–1, additional sensitivity tests including ethnicity as an adjustment variable in the regression models were conducted. The analyses showed no qualitative interactions by ethnicity. Nominal *p*-values (two-sided) less than 0.05 were considered statistically significant. Stata I/C v14.2 (StataCorp, College Station, TX, USA) and SAS 9.3 (copyright 2002–2010 by SAS Institute Inc., Cary, NC, USA) software were used for all data analyses.

RESULTS

Distribution of Hp genotype and patient characteristics

A total of 228 subjects were included in the study; 135 were adults from the PIVENS trial and 93 were children from the TONIC trial (Table 1). Among adults, 69 received VitE and 66 received placebo. Among children, 48 received VitE and 45 received placebo. For the entire study population, there was no difference between the observed distribution of Hp 1–

1, 2–1, and 2–2 (16%, 50% and 34%, respectively), compared to previously published distribution in the general Western population (16%, 48%, and 36%, respectively)[22]; p=0·91 (Table 1). In addition, Hp genotype distribution within each trial subgroup was similar to expected distribution.

The proportion of Hispanic subjects increased from Hp 2–2 to 2–1 to 1–1 (31% versus 32% versus 57% respectively, Fisher's Exact test p=0·02) (Table 2). Further analyses of data by PIVENS and TONIC showed differences in the proportion of Hispanic patients within each genotype in both adult (Supplementary Table 1) and children (Supplementary Table 2) subpopulations. Baseline anthropometric measures, laboratory data and histological features were similar among Hp genotypes with the exception of total cholesterol in adults, with Hp 2–1 adults having slightly lower levels compared to Hp 2–2 and Hp 1–1 individuals (Supplementary Table 1).

Histological improvement and resolution of NASH

When compared to their respective placebo-treated patients, VitE significantly improved liver histology only in patients with the Hp 2–2 genotype (OR 4·2; p=0·006) (Table 3). There was a step-wise decrease in the proportion of VitE-treated subjects who attained histological improvement from Hp 2–2 to 2–1 to 1–1 (51% versus 43% versus 40%), however, the interaction P-value was not significant for treatment effect for histological improvement across genotypes. VitE, compared to placebo, led to resolution of NASH in much higher proportions of Hp 2–2 patients (44% versus 12%, OR=6·2, p=0·009) and 2–1 patients (49% versus 24%, OR=3·0, p=0·01) than in 1–1 patients (28% versus 20%, OR=1·7, p=0·52) (Table 3).

Change in NAFLD activity score

Hp 1–1 subjects on VitE showed no significant change in NAS compared to placebo (−1·0±1·5 versus −0·5±2·1, adjM=−0·6, p=0·22). In contrast, Hp 2–2 patients on VitE versus placebo showed significant improvement in NAS (−2·2±1·8 versus −0·6±1·7, adjM=−1·1, p=0·001) (Table 3), as well as improvement in steatosis, inflammation and a trend towards improved hepatocellular ballooning (Figure 2). Similarly, 2–1 patients on VitE versus placebo also showed improved NAS (−1·8±2·4 versus −1·0±1·5, adjM=−1·1, p=0·002), and significantly improved steatosis, inflammation and hepatocellular ballooning (Figure 2). VitE was not associated with changes in fibrosis stage in any Hp genotype.

Improvement in liver enzymes

VitE-treated Hp 1–1 patients showed no significant change in ALT, AST, ALP, and GGT levels when compared to placebo. In contrast, there was a significantly superior effect of VitE over placebo in Hp 2–2 patients for ALT (−52·6±60·4 versus −13·6±48·8; adjM=−33·6; p<0·001), AST (−31·1±41·5 versus −1·5±24·9; adjM=−20·6; p<0·001), ALP (−29·4±47·1 versus −12·1±44·0; adjM=−16·6; p=0·04) and GGT (−17·3±25·3 versus 1·8±21·1, adjM=−18·4, p<0·001). Similarly, Hp 2–1 subjects treated with VitE versus placebo showed significant improvement in ALT (−39·3±54·0 versus −29·4±60·6, adjM=−16·9, p=0·04) and AST (−21·5±32·9 versus −12·6±36·6, adjM=−12·1, p=0·03). As shown by the interaction P-

values for trend, an incremental benefit from having Hp 2 (2–1 or 2–2) over Hp 1–1 was observed for ALT, AST, ALP and GGT with VitE treatment (Table 3).

Sensitivity analyses of adults and children

Sensitivity analyses using data from each trial (PIVENS or TONIC) were limited by the smaller number of patients per treatment group, compared to the combined analyses. However, similar results as the total group, though generally not reaching significance, were seen in each age group. Adults with the Hp 2–1 genotype who received VitE showed histologic improvement and resolution of steatohepatitis compared to placebo (Supplementary Table 3). Both Hp 2–2 and 2–1 adults showed improved NAS when treated with VitE, in contrast to 1–1 adults. Hp 2–2 and 2–1 adults showed VitE-associated improvement in liver enzymes, to varying degrees, while Hp 1–1 adults showed no improvement in liver enzymes. In pediatric patients, those with Hp 2–2 treated with VitE showed increased resolution of NASH, and improved NAS, ALT and AST compared to placebo. For all liver enzymes, there was an incremental benefit of VitE across Hp genotype in children (Supplementary Table 4).

DISCUSSION

VitE remains an important therapeutic option for those with active NASH.[8] We have previously demonstrated that only a subset of individuals with NASH respond to VitE treatment.[4,5] It is unclear why some individuals respond to VitE while others do not. In this post hoc analysis, we examined the association of Hp genotype with VitE response in nondiabetic subjects with NASH. We found that subjects with an Hp 2 allele (Hp 2–1 or 2–2) had a superior VitE effect compared to those homozygous for Hp 1 on the primary outcome of histological improvement, as well as resolution of steatohepatitis and decrease in NAS. There was also a remarkable improvement in Hp 2 containing individuals with respect to AST, ALT, ALP and GGT. These data suggest that the Hp 2 allele may confer increased likelihood of histological response following VitE treatment in those with active NASH. However, when the incremental benefit of the Hp 2 allele was compared to Hp 1 for VitE effects on these outcomes, they were significant only for AST, ALT, ALP and GGT. It is likely that the failure to demonstrate significance for the histologic outcomes was due to the small number of subjects in the subgroups, given that the study was not powered for such analyses. Thus, while these findings are highly suggestive, they must be considered exploratory.

Despite the lack of a statistically significant incremental effect of Hp 2 over Hp 1 in VitE response in some of the treatment parameters assessed, it is noteworthy that the direction of change for the histological features and liver enzymes were identical. Together with the biological rationale for Hp 2 as a biomarker of treatment response to VitE, and published literature on the subject with respect to cardiovascular parameters and outcomes,[19] the data presented herein provide a strong rationale for further studies on the role of Hp genotype in VitE-mediated treatment response in NASH. These studies would be crucial in making recommendations about the utility of Hp genotyping prior to prescribing VitE treatment for NASH; they would also provide insight on the mechanisms involved in Hp 2-mediated

modulation of VitE response. It is known that Hp 1–1 proteins are monomeric and bind hemoglobin with superior efficiency, facilitating fast and efficient clearance of free hemoglobin from the blood. In contrast, Hp 2–2 proteins form large polymers with high molecular mass and restricted distribution, limiting their ability to bind and scavenge hemoglobin and free radicals. VitE supplementation restores free radical clearance and reduces HDL oxidation.[24] How these translate into improved hepatic response in NASH is an area that requires further elucidation.

The over-representation of Hispanic individuals among the Hp 1–1 pediatric patients did not appear to contribute to the observed differences in VitE-mediated response in Hp 2 versus Hp 1 patients, since both adults and children show the same trend despite varied distribution of Hispanic individuals in these subpopulations. Sensitivity analyses with ethnicity as an adjustment variable in all regression models did not show any change in the results for the total, adult and pediatric groups; hence no qualitative interactions by ethnicity were found. Previous studies have shown that the patatin-like phospholipase domain-containing protein 3 (PNPLA3) $I^{148}M$ variant is present in up to 50% of Hispanic individuals and has been linked to more aggressive disease in this population.[25] There are no published data on the impact of PNPLA3 status on VitE treatment response. The current data further provide a rationale to evaluate the interactions between Hp and PNPLA3 genotypes in driving disease severity as well as treatment response to VitE in larger study cohorts.

Any discussion of VitE treatment for NASH and the identification of response biomarkers must be framed in the context of whether VitE confers clinically meaningful benefit to patients. Demonstration of survival benefit or decreased clinical outcomes requires large patient cohorts and long periods of follow up, posing logistical difficulties in demonstrating such benefits. Thus, progression to cirrhosis, which is the leading cause of liver-related death in NASH patients, is now generally accepted as a clinically meaningful endpoint.[26] Fibrosis progression is the hallmark of progression to cirrhosis, and VitE did not impact fibrosis for the study population as a whole or for any specific subset based on Hp genotype. It has been reported that subjects receiving VitE with a 2-point or greater improvement in NAS also experienced significantly greater fibrosis improvement compared to those with lesser degree of improvement in NAS.[27] The implication of this observation is that subjects with the Hp 2 allele who show short-term improvement in NAS may eventually demonstrate improved fibrosis, while Hp 1 patients are unlikely to have a fibrosis benefit. This however remains to be demonstrated prospectively. Over 80% of individuals in the Western population carry a Hp 2 allele, as demonstrated in our post-hoc analysis, and also as shown in the general North American population by other investigators.[14,19] Thus, the potential for therapeutic benefit of VitE, an inexpensive and widely available supplement, in a large sub-population of NASH patients carries significant implications for public health and healthcare expenditure.

In summary, this post hoc analysis of data from the PIVENS and TONIC trials suggests that subjects with Hp 2–2 and Hp 2–1 show superior response to VitE therapy for active NASH, compared to those with Hp 1–1. While this retrospective analysis was not powered to assess the incremental benefit of VitE over placebo in various Hp genotypes, the results showed trends supporting an incremental gain in VitE responsiveness in NASH patients with at least one Hp 2 allele. If future studies validate these findings, it will allow identification of NASH

patients most likely to respond to VitE prior to initiation of therapy. Such pre-selection would limit unnecessary exposure to pharmacological doses of VitE in patients with a low likelihood of response, contributing to a precision medicine-based approach to the treatment of NASH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

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Abbreviations:

Hp	haptoglobin
NASH	nonalcoholic steatohepatitis

PIVENS	Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis
TONIC	Treatment of Nonalcoholic Fatty Liver Disease In Children
NAS	nonalcoholic fatty liver disease activity score
EOT	end of treatment
ALT	alanine aminotransferase
AST	asparate aminotransferase
ALT	alkaline phosphatase
GGT	γ-glutamyl transpeptidase
NAFLD	nonalcoholic fatty liver disease
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NASH CRN	nonalcoholic steatohepatitis clinical research network
PCR	polymerase chain reaction
TAE	Tris-acetate-ethylenediaminetetraacetic acid
OR	adds ratio
adjM	adjusted mean
BMI	body mass index
HDL	high density lipoprotein
HOMA-IR	homeostasis model assessment-estimated insulin resistance
PNPLA3	patatin-like phospholipase domain-containing protein 3
VitE	Vitamin E

References

1. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012;55:2005–23. [PubMed: 22488764]
2. Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. The American journal of gastroenterology 2004;99:1497–502. [PubMed: 15307867]
3. Mota M, Banini BA, Cazanave SC, Sanyal AJ. Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. Metabolism: clinical and experimental 2016;65:1049–61. [PubMed: 26997538]
4. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362:1675–85. [PubMed: 20427778]

5. Lavine JE, Schwimmer JB, Van Natta ML, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. JAMA 2011;305:1659–68. [PubMed: 21521847]
6. Sato K, Gosho M, Yamamoto T, et al. Vitamin E has a beneficial effect on nonalcoholic fatty liver disease: a meta-analysis of randomized controlled trials. Nutrition 2015;31:923–30. [PubMed: 26059365]
7. Banini BA, Sanyal AJ. Nonalcoholic fatty liver disease: epidemiology, pathogenesis, natural History, diagnosis, and current treatment options. Clinical medicine insights Therapeutics 2016;2016:75–84
8. EASL-EASD-EASO Clinical Practice Guidelines for the Management of Non-Alcoholic Fatty Liver Disease. Obes Facts 2016;9:65–90. [PubMed: 27055256]
9. Miller ER 3rd, Pastor-Barriuso R, Dalal D Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Annals of internal medicine 2005;142:37–46. [PubMed: 15537682]
10. Klein EA, Thompson IM Jr, Tangen CM, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2011;306:1549–56. [PubMed: 21990298]
11. Abner EL, Schmitt FA, Mendiondo MS, Marcum JL, Kryscio RJ. Vitamin E and all-cause mortality: a meta-analysis. Curr Aging Sci 2011;4:158–70. [PubMed: 21235492]
12. Key TJ, Appleby PN, Travis RC, et al. Carotenoids, retinol, tocopherols, and prostate cancer risk: pooled analysis of 15 studies. The American journal of clinical nutrition 2015;102:1142–57. [PubMed: 26447150]
13. Galicia G, Ceuppens JL. Haptoglobin Functions and Regulation in Autoimmune Diseases. In: Veas F, ed. Acute Phase Proteins- Regulation and Functions of Acute Phase Proteins, 2011.
14. Bowman BH, Kurosky A. Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. Adv Hum Genet 1982;12:189–261, 453–4. [PubMed: 6751044]
15. Maeda N, Smithies O. The evolution of multigene families: human haptoglobin genes. Annual review of genetics 1986;20:81–108.
16. Delanghe JR, Langlois MR, De Buyzere ML, Torck MA. Vitamin C deficiency and scurvy are not only a dietary problem but are codetermined by the haptoglobin polymorphism. Clinical chemistry 2007;53:1397–400. [PubMed: 17644791]
17. Melamed-Frank M, Lache O, Enav BI, et al. Structure-function analysis of the antioxidant properties of haptoglobin. Blood 2001;98:3693–8. [PubMed: 11739174]
18. Asleh R, Guetta J, Kalet-Litman S, Miller-Lotan R, Levy AP. Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress in vitro and in vivo. Circulation research 2005;96:435–41. [PubMed: 15662028]
19. Blum S, Vardi M, Brown JB, et al. Vitamin E reduces cardiovascular disease in individuals with diabetes mellitus and the haptoglobin 2–2 genotype. Pharmacogenomics 2010;11:675–84. [PubMed: 20415560]
20. Koch W, Latz W, Eichinger M, et al. Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. Clinical chemistry 2002;48:1377–82. [PubMed: 12194911]
21. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21. [PubMed: 15915461]
22. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clinical chemistry 1996;42:1589–600. [PubMed: 8855140]
23. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case-control studies. Nature protocols 2011;6:121–33. [PubMed: 21293453]
24. Asleh R, Blum S, Kalet-Litman S, et al. Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2–2 genotype. Diabetes 2008;57:2794–800. [PubMed: 18599520]
25. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nature genetics 2008;40:1461–5. [PubMed: 18820647]
26. Sanyal AJ, Friedman SL, Mccullough AJ, Dimick-Santos L. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop. Hepatology 2015;61:1392–405. [PubMed: 25557690]

27. Brunt EM, Belt PH, Wilson L, et al. Progression to bridging fibrosis in non-alcoholic fatty liver disease over 4 years in the NASH CRN. Hepatology 2013;58:495A–96A.

Key points box

- No studies have investigated the association between haptoglobin (Hp) genotype and pharmacologic response to VitE in patients with NASH
- Our post hoc analysis of the PIVENS and TONIC trials showed that when treated with VitE, NASH patients with at least one Hp 2 allele had greater resolution of steatohepatitis, histologic improvement and decrease in NAS compared to those with only Hp 1 alleles
- Patients with at least one Hp 2 allele showed improved ALT, AST, ALP and GGT, with an incremental benefit from having Hp 2 (2–1 or 2–2) over Hp 1–1 when treated with VitE
- Findings are preliminary and warrant large scale studies to substantiate the utility of Hp genotyping in NASH patients prior to VitE therapy

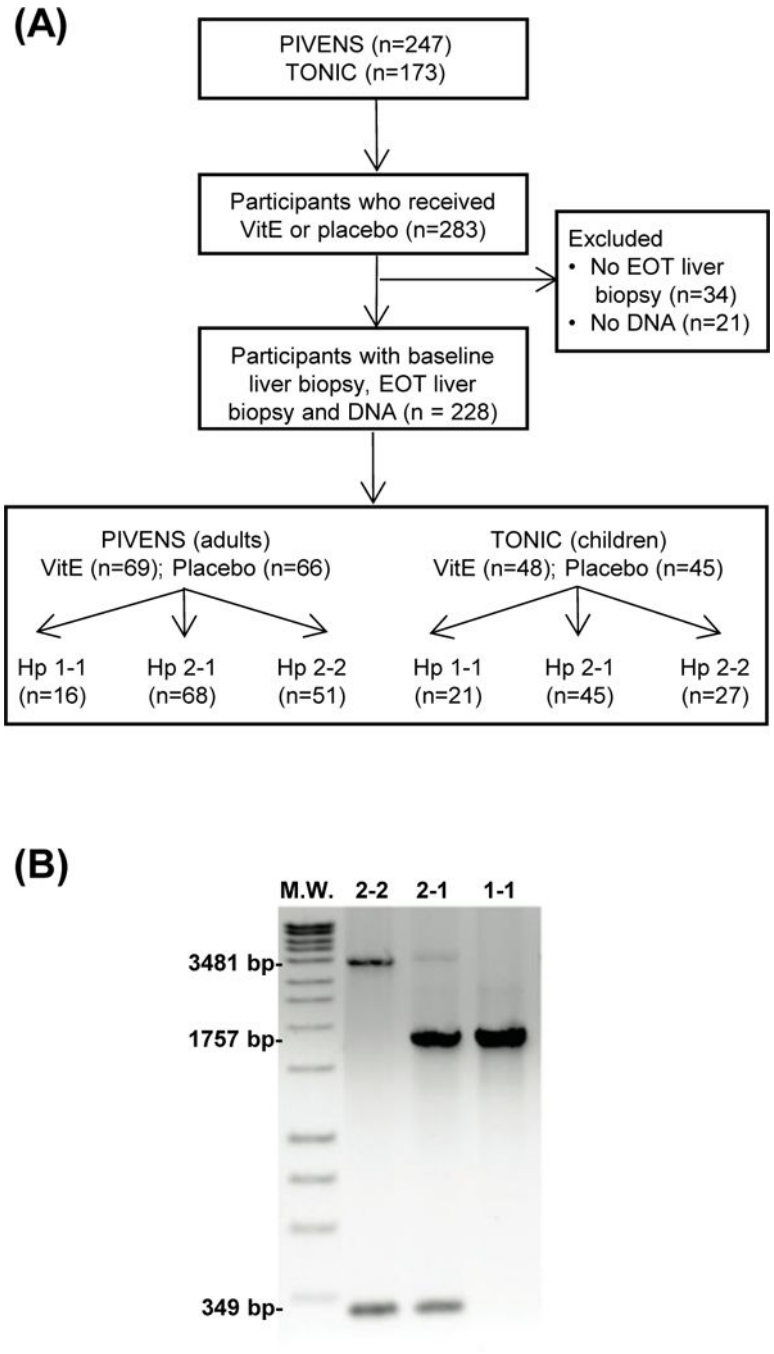

Figure 1:

(A) Study design flow diagram depicting enrollment and post-hoc exclusions for the two clinical trials, PIVENS and TONIC. (B) Distribution of haptoglobin (Hp) genotypes. Hp genotypes of study participants were determined by conventional polymerase chain reaction (PCR) using two primer sets (primers A/B; C/D). For each subject, PCR products of the two primer sets were pooled, resolved on agarose gel, and visualized under ultraviolet light. Hp 1–1 was identified by a sole 1757-bp band; Hp 2–2 by two bands at 3481-bp and 349-bp; and Hp 2–1 by bands at 1757-bp and 349-bp (with or without a faint band at 3481; and Hp

2–1 by bands at 1757-bp and 349-bp (with or without a faint band at 3481-bp).Abbreviations: Hp, haptoglobin; EOT, end of treatment; PCR, polymerase chain reaction; PIVENS, Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis; TONIC, Treatment of Nonalcoholic Fatty Liver Disease In Children; VitE, vitamin E.

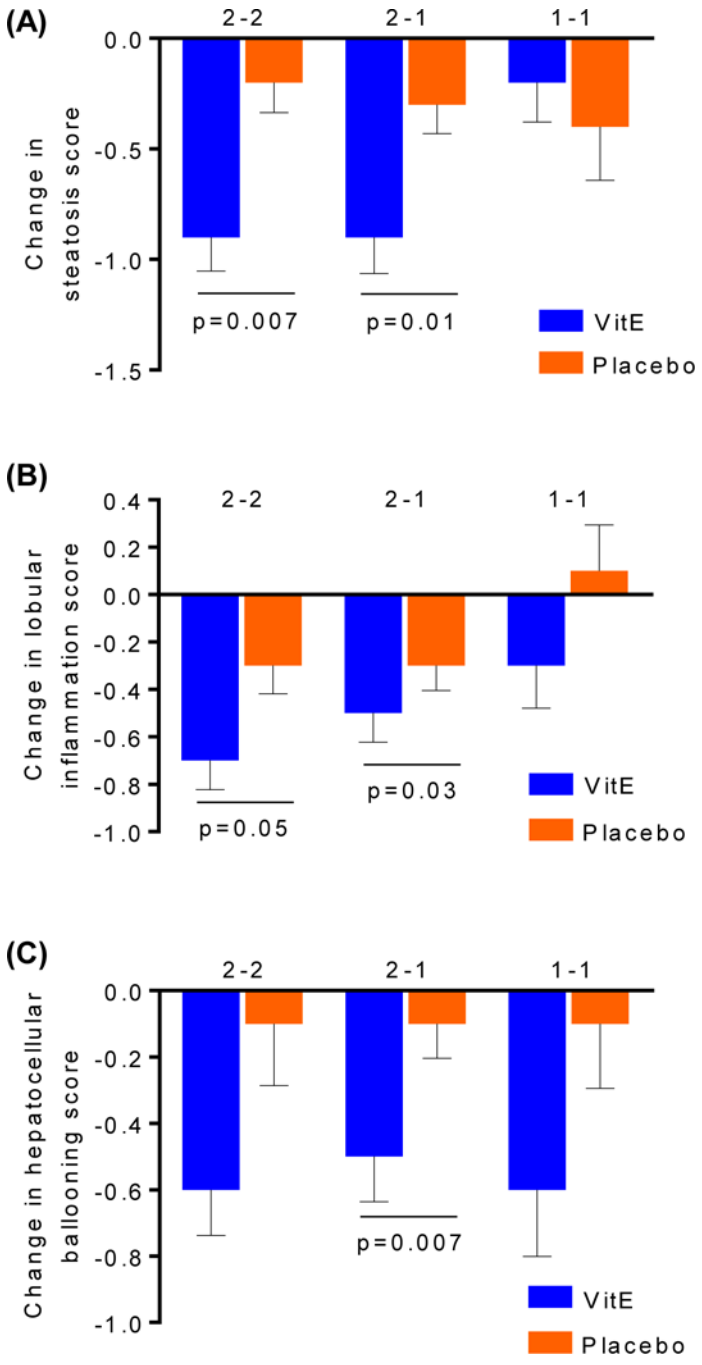

Figure 2:

Hematoxylin and eosin-stained liver sections were evaluated in a blinded manner to determine scores for steatosis (0–3), lobular inflammation (0–3) and hepatocellular ballooning (0–2) at the beginning of the original trials and at the end-of-treatment (EOT). Changes in these scores from baseline to EOT were analyzed for Hp 2–2, Hp 2–1 and Hp 1–1 patients treated with vitamin E (VitE; blue bars) versus placebo (orange bars). Changes in

steatosis (A), lobular inflammation (B) and hepatocellular ballooning (C) are shown, together with p-values comparing VitE versus placebo for each genotype.

Table 1.

Observed and expected haptoglobin genotype counts by study and treatment group in adults and children with NAFLD

		Observed Genotype Counts, n (%)				Expected Counts[2], n			
Study[1]	**Treatment**	**Hp 2–2**	**Hp 2–1**	**Hp 1–1**	**Total**	**Hp 2–2**	**Hp 2–1**	**Hp 1–1**	***p*[3]**
PIVENS	Vitamin E	29 (42%)	30 (43%)	10 (14%)	69 (100%)	25	33	11	0·85
	Placebo	22 (33%)	38 (58%)	6 (9%)	66 (100%)	24	32	10	0·45
TONIC	Vitamin E	14 (29%)	24 (50%)	10 (21%)	48 (100%)	17	23	8	0·74
	Placebo	13 (29%)	21 (47%)	11 (24%)	45 (100%)	16	22	7	0·58
Total	Vitamin E	43 (37%)	54 (46%)	20 (17%)	117 (100%)	42	56	19	0·97
	Placebo	35 (32%)	59 (53%)	17 (15%)	111 (100%)	40	53	18	0·73
All patients	--	78 (34%)	113 (50%)	37 (16%)	228 (100%)	82	109	37	0·91

[1]PIVENS, Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis. TONIC, Treatment of Nonalcoholic Fatty Liver Disease In Children.

[2]Prevalence of haptoglobin (Hp) genotypes in Western populations is 16% Hp 1–1, 48% Hp 2–1, and 36% Hp 2–2.

[3]*p*-value calculated using Fisher's exact test.

There is no evidence to reject the assumption under the Hardy-Weinburg Equilibrium (HWE) implying that the patients in these samples are from Western populations.

Table 2.

Baseline characteristics at enrollment of adult and pediatric patients by haptoglobin genotype

Characteristics	Haptoglobin genotype[1] Hp 2–2 N=78	Hp 2–1 N=113	Hp 1–1 N=37	p[2]
Treatment				0·62
Vitamin E	43 (55%)	54 (48%)	20 (54%)	
Placebo	35 (45%)	59 (52%)	17 (46%)	
Demographic characteristics				
Age	35·1 (18·9)	33·8 (19·2)	28·2 (20·0)	0·18
Sex: male	42 (54%)	56 (50%)	25 (68%)	0·16
Ethnicity: Hispanic	24 (31%)	36 (32%)	21 (57%)	**0·02**
Race:				0·95
White	64 (84%)	89 (82%)	29 (83%)	
Black	2 (3%)	2 (2%)	0 (0%)	
Other[3]	10 (13%)	17 (16%)	6 (17%)	
Anthropometric measures				
BMI (kg/m^2)	34·0 (6·9)	34·0 (6·5)	34·2 (5·7)	0·99
Laboratory measures				
Glucose (mmol/L)	5·17 (0·73)	5·09 (0·74)	5·13 (0·56)	0·78
HOMA-IR	35·2 (25·7)	47·0 (76·4)	46·7 (53·2)	0·37
Triglycerides (mmol/L)	1·80 (0·93)	1·82 (1·16)	1·47 (0·64)	0·17
Cholesterol, total (mmol/L)	5·06 (1·08)	4·77 (0·92)	4·76 (0·91)	0·10
Cholesterol, HDL (mmol/L)	1·09 (0·30)	1·06 (0·27)	1·03 (0·27)	0·51
Alanine aminotransferase (U/L)	97·4 (62·2)	95·4 (60·2)	104·4 (57·0)	0·74
Asparate aminotransferase (U/L)	62·6 (38·7)	63·3 (33·7)	66·1 (41·5)	0·89
Alkaline phosphatase (U/L)	137·0 (103·0)	135·5 (92·7)	154·7 (81·2)	0·55
γ-glutamyl transpeptidase (U/L)	54·0 (42·4)	51·5 (33·7)	52·6 (27·6)	0·89
Histological measures				
Definite steatohepatitis	49 (63%)	68 (60%)	26 (70%)	0·56
Total NAFLD activity score (0–8)	5·0 (1·4)	4·7 (1·4)	4·9 (1·6)	0·38
Steatosis grade (0–3)	2·1 (0·8)	2·0 (0·9)	2·0 (1·0)	0·90
Lobular inflammation score (0–3)	1·8 (0·7)	1·6 (0·7)	1·7 (0·7)	0·20
Hepatocellular ballooning score (0–2)	1·2 (0·8)	1·1 (0·8)	1·2 (0·8)	0·62
Fibrosis stage (0–4)	1·4 (1·1)	1·5 (1·1)	1·4 (0·9)	0·83

[1] Adult and pediatric patients are from the PIVENS and TONIC trials, respectively. All patients analyzed had paired baseline and end of treatment biopsies and DNA. Total number of patients is 228.

Data are No. (%) or mean (SD).

[2] *p*-value calculated using Fisher's exact test for categorical characteristics and ANOVA for continuous characteristics.

[3] 9 patients refused to report a race; however, 8 of the 9 identified as Hispanic.

Abbreviations: BMI, body mass index; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; Hp, haptoglobin; NAFLD, nonalcoholic fatty liver disease

Table 3.

Changes in histological features and liver enzymes after 96 weeks of treatment in adult and pediatric patients by Haptoglobin genotype comparing Vitamin E and Placebo groups

Haptoglobin genotype	No. improved/total No. (%) or mean change from baseline ± SD		OR or mean change VitE vs placebo[1]	pEvPl[1]	Interaction P[2]	
	VitE	Placebo			Hp	Trend
Histological Outcomes						
Histologic improvement[3]						
2–2	22/43 (51%)	7/35 (20%)	4·2	**0·006**		
2–1	23/53 (43%)	17/59 (29%)	2·0	0·10	0·50	n/a
1–1	8/20 (40%)	3/17 (18%)	3·5	0·12		
Resolution of NASH[4]						
2–2	18/41 (44%)	3/26 (12%)	6·2	**0·009**		
2–1	23/47 (49%)	13/54 (24%)	3·0	**0·01**	0·50	0·30
1–1	5/18 (28%)	3/15 (20%)	1·7	0·52		
NAFLD Activity Score[5]						
2–2	−2·2 ± 1·8	−0·6 ± 1·7	−1·1	**0·001**		
2–1	−1·8 ± 2·4	−0·6 ± 1·8	−1·1	**0·002**	0·56	0·40
1–1	−1·0 ± 1·5	−0·5 ± 2·1	−0·6	0·22		
Fibrosis stage[5]						
2–2	−0·5 ± 1·0	−0·2 ± 0·8	−0·3	0·16		
2–1	−0·3 ± 1·1	−0·2 ± 1·1	−0·1	0·67	0·63	n/a
1–1	−0·3 ± 1·2	0·2 ± 1·2	−0·4	0·36		
Liver enzymes (U/L)[5]						
Alanine aminotransferase						
2–2	−52·6 ± 60·4	−13·6 ± 48·8	−33·6	**<0·001**		
2–1	−39·3 ± 54·0	−29·4 ± 60·6	−16·9	**0·04**	**0·03**	**0·008**
1–1	−13·5 ± 59·5	−33·6 ± 60·4	16·4	0·41		
Aspartate aminotransferase						
2–2	−31·1 ± 41·5	−1·5 ± 24·9	−20·6	**<0·001**		
2–1	−21·5 ± 32·9	−12·6 ± 36·6	−12·1	**0·03**	**0·003**	**0·002**
1–1	−4·7 ± 32·3	−22·4 ± 43·0	16·0	0·13		
Alkaline phosphatase						
2–2	−29·4 ± 47·1	−12·1 ±44·0	−16·6	**0·04**		
2–1	−26·8 ± 57·7	−23·7 ± 44·6	−3·3	0·70	**0·03**	**0·01**
1–1	−12·9 ± 41·8	−38·5 ± 56·5	25·3	0·10		
γ-glutamyl transpeptidase						
2–2	−17·3 ±25·3	1·8 ± 21·1	−18·4	**<0·001**		
2–1	−7·5 ± 24·6	−4·7 ± 31·2	−5·0	0·34	0·12	**0·05**
1–1	−3·6 ± 27·6	−1·7 ± 22·3	−0·5	0·96		

[1]Odds ratios (OR) and *p*-values were calculated using logistic regression, regressing the binary outcome (improvement, resolution) on the treatment group for each haptoglobin genotype; *p*-values and mean changes from baseline were calculated using ANCOVA, regressing change from baseline to 96 weeks on treatment group and baseline value of the outcome for each haptoglobin genotype. All regressions were adjusted for an indicator of age (adult vs pediatric).

[2]Interaction P-values calculated using logistic regression models for binary outcomes and linear regression models for continuous outcomes, regressing haptoglobin genotype (3-category), age indicator, and the treatment group by haptoglobin genotype, assuming the genotypic association model. In calculating the P-values for the additive association model (trend), haptoglobin genotype was treated as an ordinal variable where Hp 1–1=0, Hp 2–1=1, and Hp 2–2=2. "n/a" denotes that the additive model was not appropriate due to violating model assumptions.

[3]Histologic improvement defined as a decrease of at least 2 points in the NAS score and no worsening of fibrosis from baseline to end of treatment

[4]For resolution of NASH, 27 patients with NAFLD but not NASH on the baseline biopsy were excluded from the analyses

[5]Total N=228; Hp 2–2 vitamin E, *n*=43; Hp 2–2 placebo, *n*=35; Hp 2–1 vitamin E, *n*=54; Hp 2–1 placebo *n*=59; Hp 1–1 vitamin E, *n*=20; Hp 1–1 placebo, *n*=17