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NOS1AP genetic variation is associated with impaired healing of diabetic foot ulcers and diminished response to healing of circulating stem/progenitor cells

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Abstract

It is unclear why many with diabetes develop foot ulcers (DFU) and why some do not heal. It could be associated with genetic variation. We have previously shown that *NOS1AP* variation is associated with lower extremity amputation in those with diabetes and that circulating stem progenitor cell concentration (SPC) is associated with impaired foot ulcer healing in those with diabetes. The goal of this study was to determine if *NOS1AP* variation is associated with impaired wound healing and with SPC mobilization in those with DFU. In longitudinal cohort study we demonstrate that *NOS1AP* variants rs16849113 and rs19649113 are associated with impaired wound healing and with SPC mobilization in those with DFU. We believe that further study of NOS1AP is merited and that it *NOS1AP* might be associated with a functional impairment.

Brief Report

Approximately 20% of those with diabetes mellitus will develop a diabetic foot ulcer (DFU) during their lifetime, and having a DFU is the major risk factor for a lower extremity amputation (LEA).(1) It is unclear why the majority of diabetics do not develop DFUs. It is possible that only a subset of individuals are genetically predisposed to difficulties with wound repair, possibly due to an interaction between a gene and the diabetic state. The role of genetic variation in the onset of DFU or LEA has not been well studied.(2)

Because DFUs are common and increase the likelihood of LEA, we previously studied a well-defined longitudinal cohort study called the Chronic Renal Insufficiency Cohort

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(CRIC), which includes nearly 1300 individuals with diabetes and, unlike most other genetic databases of those with diabetes, LEA was included as an outcome measure (unfortunately foot ulcer was not captured by CRIC).(2) In that study, we noted that gene variants of nitric oxide synthase 1 adaptor protein (*NOS1AP;* OMIM:60551) were associated with LEA; rs16849113 in those who self-reported being African-American and rs19649113 in those who self-reported being white.(2) The *NOS1AP* gene produces a protein called capon that was initially shown to interact with NOS1, regulating nitric oxide (NO) production at post-synaptic sites in neurons.(3) NO, a gaseous free radical, is a messenger molecule with diverse functions throughout the body. NO has been shown to be a cell signaling molecule with varied effects on angiogenesis, stem progenitor cell (SPC) mobilization, and wound repair.(4, 5)

We also recently demonstrated that there is an association between SPC and the healing of DFUs.(6) Briefly, this longitudinal cohort study demonstrated that subjects with a DFU who healed (n = 37) versus those who did not (n = 63) after receiving routine care by the 16^{th} week of observation had an increased number of circulating SPCs.(6) The adjusted odds ratios (OR) of association between the SPC concentration at study entry and at the first week office visit were significantly associated with healing; 2.7 (1.1, 6.3), p=0.028 and 4.7 (1.8, 12.0) p=0.001, respectively.(6) These results were adjusted for patient age and wound size at entry into the study.

Based on this prior work, we hypothesized that *NOS1AP* variation may be associated with wound healing and with SPC mobilization in those with a DFU. We studied a longitudinal cohort of subjects enrolled in an ongoing study designed to replicate (“replication study”) a previously reported association between circulating SPCs and the likelihood that a subject with a DFU will heal.(6) Subjects were enrolled at two study sites, Greater Baltimore Medical Center and the Wound Care Center at the Penn Presbyterian Medical Center. Enrollment criteria have been previously published.(6) Briefly, all subjects had type 2 diabetes, a plantar surface ulcer, neuropathy, an ankle brachial ratio of >0.65, and the absence of a history of kidney disease requiring dialysis. All subjects enrolled in this study received standard wound care for their diabetic foot ulcer as determined by their provider. Basic demographic data and wound data was obtained. The study included no therapeutic intervention and all subjects signed an informed consent provided by either University of Pennsylvania or Greater Baltimore Medical Center.

The blood specimens used in this study were discarded from the “replication study”. Permission to use the discarded biomaterials was obtained by the University Maryland Institutional Review Board, which was the site where the materials were stored. DNA was purified by GenoFIND laboratory from whole blood initially obtained in Cyto-chex BCT tubes using Gentra Puregene blood kit. Based on previous work, two single nucleotide polymorphisms (SNP), rs16849113 and rs19649113, were assayed using TaqMan technique by the GenoFIND laboratory.(2) SPCs were assayed by flow cytometry based on CD34 and CD45 surface markers and determined to be CD-34+/CD-45-dim following published methods.(6)

Initial statistical comparisons where conducted with t-tests and chi square tests. Statistical assessment of the association between SPCs and *NOS1AP* gene variation was conducted using linear regression. Impaired healing was assessed as time to healing using a Cox proportional hazards model and logistic regression was used to compare those that healed to those who did not.

At the time of this investigation 49 subjects had been enrolled in the "replication study".(6) DNA was available from 47 (95.9%) of these subjects. On average, DNA purification yielded 50 (sd 6.8) μg per subject (PicoGreen method). The basic demographic and subject evaluations are presented in Table 1. In this cohort of 47 subjects, 38% healed by the 16th week of care. We did not find a difference in SPC concentration at baseline between those who healed and those who did not. However, we found evidence to support our previous findings that circulating SPCs number per μl of blood at the week 1 visit was greater among those with a healed wound by the 16th week of care (p=0.039) (Table 1). In addition, change in SPC concentration between the baseline and week 1 visit was significantly associated with healing by the 16th week of care (p=0.0008) (Table 1).

We evaluated rs16849113 in those who self-reported being African-American and rs19649113 in those who self-reported being white based on a previous study.(2) To evaluate the overall effect of *NOS1AP* variation, we created a composite variant based on the race appropriate variant. The *NOS1AP* minor variant frequency per allele was 48.1% (26/54) for rs16849113 among those who are African-Americans, 25.7% (9/34) for rs1963645 among those who are white, and 38.9% (35/90) for the composite variant in our full cohort. *NOS1AP* variation was significantly associated with the time to healing (HR 0.30 (0.16, 0.82) p=0.015) (Table 2). We found that the concentration of circulating SPCs was significantly associated with *NOS1AP* variation (at baseline p=0.003 and at week 1 p=0.002) (Table 2). The baseline SPC concentration was also associated with actual genotype (wildytype(mean): 0.96 SPC/μl(SD 0.63), heterozygote: 0.60 (SD 0.26) and homozygote: 0.67 (SD 0.48, p=0.001).

Our results support previous findings that an association exists between *NOS1AP* and LEA (2) and that circulating SPCs concentration is associated with DFU healing.(6) Moreover, new results expand on previous knowledge by showing that *NOS1AP* variation is significantly associated with the time required for a DFU to heal and that SPCs mobilization is associated with *NOS1AP* variation. Specifically those with *NOS1AP* SNP rs16849113 who are African-American or rs19649113 who are white heal more slowly in that they are about 70% less likely to heal at any point in time and are about 50% less likely to heal by the 16th week of care.

Our previous work that identified *NOS1AP* variation used data from a cohort study designed to longitudinally evaluate the progression of renal insufficiency.(2) The outcome we analyzed was LEA. Because the majority of those with diabetes and an LEA, previously had a DFU, we hypothesized that *NOS1AP* variation would also have an effect on whether a DFU might heal.(1) In fact, reanalyzing data from our previous CRIC analysis using the *NOS1AP* composite reported in our current study revealed that *NOS1AP* genetic variation is highly associated with LEA (1.68 (1.28, 2.21); $p< 1.00 \times 10^{-10}$). This effect likely is specific

to those with diabetes in that no association was noted for LEA and *NOS1AP* variation in those without a history of diabetes (p=0.347).(2) Our new data replicate our previous findings and go further to demonstrate that *NOS1AP* variation is associated with impaired healing in those with diabetes.

It is reasonable to consider that the association between SPC mobilization and *NOS1AP* variation relates to biochemical role(s) for the *NOS1AP* coded protein, capon (carboxy-terminal PDZ ligand of type 1 nitric oxide synthase). Capon's influence on NOS1 activity is thought to be the basis for associations of *NOS1AP* variation with a number of neurological and cardiac disorders, but there is also an association with severe chronic rhinosinusitis.(7, 8) As all three NOS isoforms influence a variety of stem cell functions,(5) capon influence may be mediated by its action on NOS1. There could also be other effects. Three variants of capon have been described that range from about 80 to 500 amino acids in composition.(9) Capon is present in many different cells/organs and its subcellular localization differs, seemingly, due to alterations in its N- and C-terminal regions and to the bridging proteins with which capon interacts.(10, 11) *NOS1AP* variants have been associated with the incidence of diabetes among users of various medications, as well as with the therapeutic efficacy of some medications used to treat type 2 diabetes.(12–14) *NOS1AP* variants may play a small, inconsistent role in diabetes onset in the general population.(15)

Over the past 20 years, advances in therapies to treat DFU as well as our understanding of why individuals with DFU may not heal have been limited. In our study we confirm that *NOS1AP* variation is associated with impaired healing in individuals with diabetes. This is one of the first genes to be consistently identified to be associated with impaired healing in those with diabetes. It is possible that the mechanism of action is due to effects of capon on NO mediated SPC mobilization and function. Probing *NOS1AP gene* variations associated with high risk for impaired healing offers an exceptional opportunity to characterize the functional implications of capon and to potentially consider new therapeutics.

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References

1. Margolis DJ, Jeffcoate WJ. Epidemiology of foot ulceration and amputation-can global variation be explained? Medical Clinics of North America. 2013; 97:791–805. [PubMed: 23992892]
2. Margolis DJ, Gupta J, Thom SR, Townsend RR, Kanetsky P, Hoffstad O, et al. Diabetes, lower extremity amputation, loss of portective sensation, and NOS1AP in the CRIC study. Wound Rep and Reg. 2013; 21:17–24.
3. Courtney MJ, Li LL, Lai YY. Mechanisms of NOS1AP action on NMDA receptor-nNOS signaling. Front Cell Neurosci. 2014; 8:252. [PubMed: 25221472]
4. Isenberg JS, Ridnour LA, Espey MG, Wink DA, Roberts DD. Nitric oxide in wound healing. Microsurgery. 2005; 25:442–51. [PubMed: 16044466]
5. Wang W, Lee Y, Lee CH. Effects of nitric oxide on stem cell therapy. Biotechnology Advances. 2015; 33:1685–96. [PubMed: 26394194]

6. Thom SR, Hampton M, Troiano MA, Mirza Z, Malay DS, Shannon S, et al. Measurements of CD34+/CD45-dim Stem Cells Predict Healing of Diabetic Neuropathic Wounds. Diabetes. 2016; 65(2):486–97. [PubMed: 26487786]

7. Zhang Y, Endam LM, Filali-Mouhim A, Bosse Y, Castano R, Desrosiers M. Polymorphisms in the nitric oxide synthase 1 gene are associated with severe chronic rhinosinusitis. Am J Rhinol & Allergy. 2011; 25:e49–e54. [PubMed: 21679499]

8. Jamshidi Y, Nolte IM, Dalageorgou C, Zheng D, Johnson T, Bastiaenen R, et al. Common variation in the NOS1AP gene is associated with drug-induced QT prolongation and ventricular arrhythmia. J Am Coll Cardiol. 2012; 60(9):841–50. [PubMed: 22682551]

9. Li LL, Melero-Fernandez de Mera RM, Chen J, Ba W, Kasri NN, Zhang M, et al. Unexpected Heterodivalent Recruitment of NOS1AP to nNOS Reveals Multiple Sites for Pharmacological Intervention in Neuronal Disease Models. J Neurosci. 2015; 35(19):7349–64. [PubMed: 25972165]

10. Wang J, Jin L, Zhu Y, Zhou X, Yu R, Gao S. Research progress in NOS1AP in neurological and psychiatric diseases. Brain Res Bull. 2016; 125:99–105. [PubMed: 27237129]

11. Clattenburg L, Wigerius M, Qi J, Rainey JK, Rourke JL, Muruganandan S, et al. NOS1AP Functionally Associates with YAP To Regulate Hippo Signaling. Mol Cell Biol. 2015; 35(13): 2265–77. [PubMed: 25918243]

12. Becker ML, Aarnoudse AJ, Newton-Cheh C, Hofman A, Witteman JC, Uitterlinden AG, et al. Common variation in the NOS1AP gene is associated with reduced glucose-lowering effect and with increased mortality in users of sulfonylurea. Pharmacogenet Genomics. 2008; 18(7):591–7. [PubMed: 18551039]

13. Chu AY, Coresh J, Arking DE, Pankow JS, Tomaselli GF, Chakravarti A, et al. NOS1AP variant associated with incidence of type 2 diabetes in calcium channel blocker users in the Atherosclerosis Risk in Communities (ARIC) study. Diabetologia. 2010; 53(3):510–6. [PubMed: 19943157]

14. Wang T, Wang Y, Lv DM, Song JF, Lu Q, Gao X, et al. Effects of NOS1AP rs12742393 polymorphism on repaglinide response in Chinese patients with type 2 diabetes mellitus. Pharmacotherapy. 2014; 34(2):131–9. [PubMed: 24338736]

15. Hu C, Wang C, Zhang R, Ng MC, Bao Y, Wang C, et al. Association of genetic variants of NOS1AP with type 2 diabetes in a Chinese population. Diabetologia. 2010; 53(2):290–8. [PubMed: 19937226]

Table 1

Study demographics and progenitor/stem cells (SPC) concentration per μl of blood. Data summarized as means or percentages. Comparisons made by T-Test or chi square. F= female, AA-African American; SD=standard deviation

Covariate	Overall (N=47)	Not Healed by week 16 (N=29)	Heal by week 16 (N=18)
Age	59.3 (SD 12.0)	60.2 (SD 12.7)	57.8 (SD 2.6) P=0.516
Gender (F)	70.2 %	72.4%	66.7% P=0.675
Race (AA)	55.3%	51.7%	61.1% P=0.529
Wound size at baseline (mm)	3.2 (SD 3.8)	3.9 (SD 4.3)	2.3 (SD 2.8) P=0.181
SPC at baseline	0.9(SD 0.6)	1.0 (SD 07)	0.8 (SD 0.5) P=0.1612
SPC at week 1	0.9 (SD 0.08)	0.8 (SD 0.52)	1.1 (SD 0.64) P=0.039
Change SPC baseline to week 1	0.0 (SD 0.6)	−0.2 (SD 0.6)	0.4 (SD 0.5) P=0.0008

Table 2

Effect estimates of healing outcome or SPC concentration per μl of blood by NOS1AP variant.

Comparison	rs16849113 African Americans (N=26)	rs1963645 Whites (N=21)	Composite (N=47)	Composite (fully adjusted) (N=47)
Time to heal[^]	0.42(0.17,1.03) P=0.057	0.34(0.04,1.57) P=0.282	0.42(0.20,0.91) P=0.028	0.36(0.16,0.82) P=0.015
Healed by 16th week[~]	0.54(0.20,1.50) P=0.242	0.61(0.12,2.99) P=0.545	0.62(0.28,1.35) P=0.228	0.47(0.19,1.15) P=0.099
SPC concentration per μl of blood at baseline[*]	−0.12(−0.48,0.25) P=0.509	−0.80(−1.17, −0.44) P=0.003	−0.36(−0.62, −0.12) P=0.005	−0.42(−0.69, −0.15) P=0.003
SPC concentration per μl of blood at week 1[*]	−0.20(−0.58,0.18) P=0.293	−0.74(−1.11, −0.36) P=0.001	−0.36(−0.62, −0.10) P=0.009	−0.45(−0.73, −0.17) P=0.002
The change in SPC concentration per μl of blood from baseline to week 1[*]	−0.10(−0.48,0.28) P=0.590	0.08(−0.30,0.44) P=0.695	0.01(−0.23,0.24) P=0.968	−0.03(−0.28,0.23) P=0.822

All models assumed an additive genetic model.

[^] hazard rate ratio;

[~] odds ratio;

[*] linear regression coefficient.

Fully adjusted-age, gender, wound size at baseline.