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ARTICLE

# Small fibre dysfunction, microvascular complications and glycaemic control in type 1 diabetes: a case–control study

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**Abstract**
*Aims/hypothesis* The aim of this study was to determine the influence of microvascular disease on C-fibre function in patients with type 1 diabetes of moderate duration.
*Methods* The axon-reflex flare area induced on the dorsum of the foot by local skin heating to 47°C was measured with a laser Doppler imager (LDI) in sex-, age- and height-matched groups with type 1 diabetes, with and without microvascular disease (MV+ and MV−, respectively) and in healthy controls (HC). Each group consisted of 24 individuals and all were free from clinical neuropathy (neuropathy disability score <3 and Toronto clinical neuropathy score <5).
*Results* LDI flare (LDIflare) was reduced in MV+ compared with HC (5.1±1.8 vs 10.0±3.1 $cm^2$, $p<0.0001$) and MV− groups (9.9±2.9 $cm^2$, $p<0.0001$). MV− and HC groups did not differ. There was no difference in diabetes duration between MV− and MV+ groups (17.5±5.7 and 20.1±5.2 years, $p=0.21$) nor current $HbA_{1c}$ (MV− 8.0±1.2% [64±10 mmol/mol]; MV+ 8.0±0.9% [64±9 mmol/mol], $p=0.53$); neither variable correlated with flare size. In contrast, duration-averaged $HbA_{1c}$ was higher in the MV+ group (8.6±0.9% [70±9 mmol/mol] vs 7.6±0.6% [60±7 mmol/mol], $p<0.001$) and correlated with LDIflare size ($r=-0.50$, $p<0.001$). Triacylglycerols were higher in MV+ compared with MV− (1.23±0.121 vs 0.93±0.7 mmol/l, $p=0.04$), but other metabolic variables did not differ between the groups.
*Conclusions/interpretation* We have shown that glycaemic burden and the presence of microvascular complications are associated with small fibre dysfunction in type 1 diabetes.

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**Keywords** Laser Doppler imager · LDIflare · Microvascular skin response · Neuropathy, somatic · Small fibre neuropathy · Type 1 diabetes

**Abbreviations**

| | |
|---|---|
| HC | Healthy controls |
| IENFD | Intra-epidermal nerve fibre density |
| LDI | Laser Doppler imager |
| LDIflare | Laser Doppler imager flare |
| MV− | Without microvascular disease |
| MV+ | With microvascular disease |
| NDS | Neuropathy disability score |
| TCNS | Toronto clinical neuropathy score |
| VPT | Vibration perception threshold |

## Introduction

Diabetic peripheral neuropathy affects up to 50% of individuals with diabetes and results in significant morbidity, mortality and healthcare costs [1]. There is increasing evidence that small unmyelinated nerve fibres that mediate pain and temperature (C-fibres) are involved early in the development of neuropathy in type 2 diabetes [2–5]. Indeed, Smith et al. showed that the neuropathy associated with impaired glucose tolerance (IGT) primarily affects small fibres and is similar to early diabetes-associated neuropathy [6]. Furthermore, in idiopathic painful peripheral neuropathy, a condition primarily affecting small nerve fibres, metabolic dysregulation has been implicated [7, 8]. In support of these observations, we have recently demonstrated impaired C-fibre function in individuals with type 2 diabetes free from clinical neuropathy and in individuals with IGT using a relatively new method, the laser Doppler imager

flare (LDIflare) technique [3, 9, 10]. This involves heating the skin on the dorsum of the foot to 44°C and measuring the size of the axon-reflex-mediated flare using a laser Doppler imager (LDI). The neurogenic nature of the LDIflare has been confirmed by demonstrating that the LDIflare is abolished by prior application of eutectic mixture of local anaesthetics (EMLA) cream over the area to be heated [3, 11]. The LDIflare also correlates well with dermal nerve fibre density ($r$=0.57, $p$<0.0001) and intra-epidermal nerve fibre density ($r$=0.57, $p$<0.0001) and is more sensitive than quantitative sensory testing [9, 10].

In the previously described study of individuals with IGT, we also included a cohort of individuals with a long duration of type 1 diabetes matched with the IGT group for age and the absence of neuropathy and microvascular disease. Expecting to find an even greater impairment in C-fibre function on account of their greater glycaemic burden we were surprised to find normal small fibre function [9]. These observations suggested the possibility of different aetiopathogenic mechanisms for neuropathy in the two forms of diabetes. We hypothesised that in type 2 diabetes factors associated with the metabolic syndrome other than hyperglycaemia may be causative, whereas in type 1 diabetes the link may be the presence of microangiopathy. However, our previous study only included individuals free from microvascular complications. The present study was therefore undertaken specifically to establish whether there is a relationship between C-fibre function and microangiopathy in type 1 diabetes.

## Methods

In this case-controlled study, LDIflares were measured in sex-, age-, height- and weight-matched groups of people with type 1 diabetes with and without structural microvascular disease (MV+ and MV−, respectively; $n$=24 in each group) and healthy controls (HC; $n$=24); none of the participants had established clinical neuropathy. The HC group was confirmed by oral glucose tolerance testing. Microvascular disease was considered to be present if there was: (1) bilateral background retinopathy for at least 2 years' duration (confirmed by digital retinal photographs and assessed by nationally accredited retinal screeners); (2) pre-proliferative or proliferative retinopathy or past laser treatment; (3) presence of microalbuminuria (urine albumin:creatinine ratio >2.5 mg/mmol for males and >3.5 mg/mmol for females) in two out of three early morning spot urine samples within a 3 to 6 month period and present for at least 2 years; or (3) all of the above. Clinical neuropathy was excluded using the neuropathy disability score (NDS; NDS <3) [12] and the Toronto clinical neuropathy score (TCNS <5) [13]. Individuals also underwent measurement of vibration perception threshold (VPT) with a neurothesiometer (Horwell Instruments, Nottingham, UK). In all individuals the VPT was below the 75th centile for their age. Those with type 1 diabetes had presented with a typical history and required insulin treatment from diagnosis. Individuals with ankle brachial pressure indices of <1.0, current smokers and those unable to increase their maximum hyperaemia (LDImax) to more than four times the resting blood flow were excluded. Only one recruited individual was excluded for this reason. Participants with the metabolic syndrome defined using the International Diabetes Federation criteria were excluded [14]. All subjects in the study gave informed consent; ethics approval was obtained from the Essex 2 Ethics Committee and the study was carried out in accordance with the Declaration of Helsinki as revised in 2000.

*Assessment of the LDIflare* The exact method used for the LDIflare technique is published in detail elsewhere [3]. It involves skin heating to 44°C for 20 min. In the present study we have used a modification of our previous method that involves heating the skin for 6 min in a stepwise fashion: 44°C for 2 min, 46°C for 1 min and finally 47°C for 3 min. This not only reduces the heating time but produces larger and more consistent flares [15]. Skin heating was commenced only after acclimatisation in a temperature-controlled room (25±1°C) and after the temperature of both the dorsum of the foot and the hallux exceeded 30°C. A circular heating probe of 1 $cm^2$ area was then applied to the foot in an area on the dorsum of the foot approximately 2–3 cm proximal to the first and second metatarsal heads. After heating, the area was immediately scanned using an LDI (Moor Instruments, Axminster, UK). The flare area was identified on the computer as the area with hyperaemic response >300 perfusion units (PU). This was measured using Moor V 5.3 software. Additionally, the LDImax in the skin immediately beneath the heater was also measured using the imager. Unlike the flare, LDImax is mediated by non-neurogenic means and reflects endothelial function [3, 11]. The size of the LDIflare depends on C-fibre function and the underlying skin small fibre neural network and extent of interconnections. The intensity of the hyperaemic response depends on the ability of the microvasculature to vasodilate. Impaired microvascular function will not affect the flare size provided the hyperaemic response exceeds the 300 PU (approximately 4× baseline). This newer PU definition relates to a software upgrade by Moor Instruments and the PU are higher than those previously published by our unit.

*Duration-averaged $HbA_{1c}$* The $HbA_{1c}$ readings from diagnosis encompassing the entire duration of diabetes were available for all individuals. These were averaged to provide a duration-averaged $HbA_{1c}$ value. All individuals except one (MV+ group) had at least one reading per year (mean of 2.7 readings $year^{-1}$ $individual^{-1}$).

*Statistical analysis* Data are presented as means±SD. The means were compared sequentially with the Mann–Whitney *U* test. Variables were correlated using the Pearson coefficient. The CVs for LDIflare and LDImax were conservatively estimated at 20% (actual CVs: LDIflare, 12.5%; LDImax, 7%), and a pre-study power calculation suggested 21 participants per group would detect a 20% difference with 90% power. Multiple linear regression was carried out using the stepwise regression method. SPSS 17.0 for Windows was used for statistical analysis and data were initially collected on Microsoft Excel 2003.

## Results

Patient demographics are detailed in Table 1. There were no significant differences in sex, age, height and weight, BMI, blood pressure or VPT between any of the three groups. The duration of diabetes was lower in the MV− group, but it was not significantly different from the MV+ group (17.7±5.7 and 20.1±5.2 years, $p=0.210$; Table 1). NDS and TCNS did not differ between the groups. Although there was no significant difference in current $HbA_{1c}$ between the groups (MV− 8.0±1.2% [64±10 mmol/mol]; MV+ 8.0±0.9% [64±9 mmol/mol], $p=0.53$), duration-averaged $HbA_{1c}$ was significantly lower in the diabetic group without complications (MV− 7.6±0.6%; [60±7 mmol/mol]; MV+ 8.6±0.9% [70±9 mmol/mol], $p=0.001$). There was a significant inverse correlation between LDIflare size and duration-averaged $HbA_{1c}$, ($r=-0.50$, $p<0.001$; Fig. 1). This relationship persisted on stepwise multiple regression analysis (with LDIflare as dependent and independent variables of duration-averaged $HbA_{1c}$, age, height, weight) with $F$ statistic 14.8, $p=0.0001$.

The LDIflare was significantly lower in the MV+ group compared with HC (5.1±1.8 vs 10.0±3.09 $cm^2$, $p<0.0001$) and compared with the MV− group (9.9±2.9 $cm^2$, $p<0.0001$; Fig. 2). In contrast, the LDIflares of the MV− and HC groups were not significantly different (9.9±2.9 vs 10.0±3.1 $cm^2$, $p=0.55$). Compared with HC, the LDImax was reduced in both the MV− (834±169 vs 685±141 PU, $p=0.003$) and the MV+ groups (632±186 PU, $p<0.001$; Fig. 3). The MV+ group had a lower LDImax than the MV− group, but this was not significant (685±141 vs 632±156, $p=0.099$). There was no correlation between LDImax and LDIflare within any of the groups. Fasting triacylglycerols were higher in MV+ (1.23±0.12) group compared with MV− (0.92±0.07, $p=0.04$) but were not different from the HC (1.02±0.21, $p$=NS). There was no correlation between fasting triacylglycerols and LDIflare size within groups. There was no significant within-group correlation between LDIflare size and total cholesterol, HDL-cholesterol or LDL-cholesterol.

## Discussion

In this study, we have shown that individuals with type 1 diabetes without clinical neuropathy have evidence of C-fibre dysfunction as shown by reduced LDIflare responses and that this relates to the presence of microvascular disease.

**Table 1** Baseline demographics and clinical characteristics of the study groups

| Characteristic | HC | MV− | MV+ |
|---|---|---|---|
| *n* | 24 | 24 | 24 |
| Women (%) | 49 | 50 | 46 |
| Age (years) | 41.5±10.8 | 40.7±9.0 | 40.9±8.6 |
| Height (m) | 1.71±0.07 | 1.73±0.08 | 1.72±0.09 |
| Weight (kg) | 72.1±10.6 | 73.8±10.9 | 76.7±9.93 |
| BMI ($kg/m^2$) | 24.5±3.0 | 24.7±2.6 | 25.7±2.8 |
| Systolic BP (mmHg) | 121±12 | 121±11 | 123±13 |
| Diastolic BP (mmHg) | 73±10 | 71±8 | 74±9 |
| VPT (V) | 5.6±2.08 | 6.3±1.9 | 6.7±1.9 |
| NDS | 0.16±0.48 | 0.16±0.48 | 0.21±0.57 |
| TCNS | 0.30±0.77 | 0.38±0.95 | 0.98±1.33 |
| Duration of diabetes (years) | 0 | 17.7±5.5 | 20.1±5.2 |
| Current $HbA_{1c}$ ,% (mmol/mol) | – | 8.0±1.2 (64±10) | 8.0±0.9 (64±9) |
| Duration-averaged $HbA_{1c}$ , % (mmol/mol) | – | 7.6±0.6 (60±7) | 8.6±0.9 (70±9)*** |
| Number receiving statin treatment | 2 | 3 | 7 |
| Number receiving fibrate treatment | 0 | 0 | 0 |
| Total cholesterol (mmol/l) | 4.91±0.84 | 4.40±0.49 | 4.62±0.91 |
| Fasting triacylglycerol (mmol/l) | 1.02±0.21 | 0.92±0.07 | 1.23±0.12* |

Data are expressed as mean±SD
*$p=0.04$ and ***$p=0.001$ for MV+ vs MV−

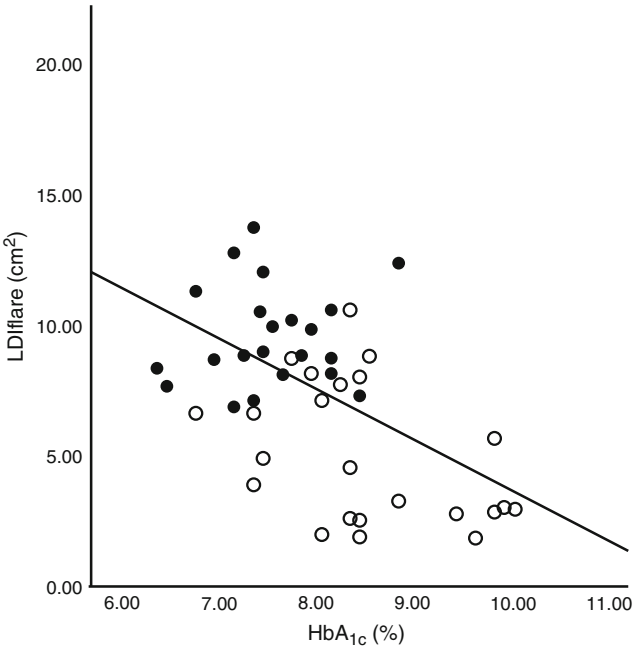


**Fig. 1** Correlation between LDIflare size and duration-averaged $HbA_{1c}$ (%) ($r=-0.50$, $p<0.001$). To convert values for $HbA_{1c}$ in % into mmol/mol, subtract 2.15 and multiply by 10.929. Black circles, MV−; white circles, MV+; solid line, fit line for total ($R^2$ linear=0.248)

Furthermore, we found a relationship between C-fibre function and glycaemic burden represented by duration-averaged $HbA_{1c}$. These findings support the hypothesis that microvascular disease is implicated in the aetiology of C-fibre dysfunction in type 1 diabetes and both are related to long-term glycaemic load. However, other factors that could explain the findings need to be considered.

It is known that age, sex and height have significant effects on the structure and function of large nerve fibres [16, 17]. The same may be the case for small fibre function.

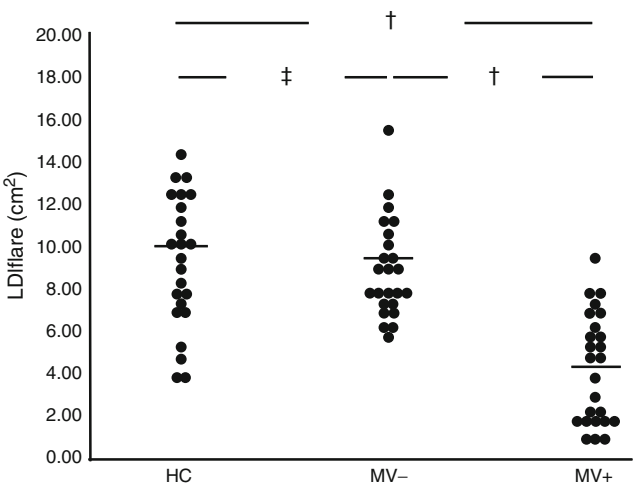


**Fig. 2** LDIflare areas in the three study groups. LDIflare was reduced in the MV+ group compared with the HC group ($^{\dagger}p<0.0001$) and MV− group ($^{\dagger}p<0.0001$); $^{\ddagger}p=0.55$ for HC vs MV−

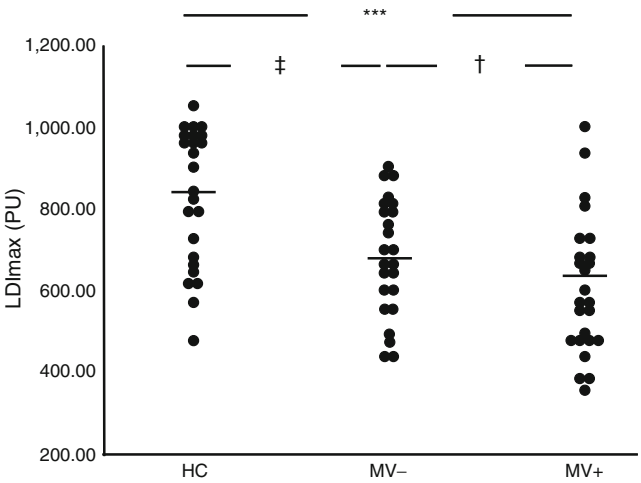


**Fig. 3** LDImax in the three study groups. LDImax was reduced in MV+ and MV− groups compared with the HC group (***$p<0.001$) but was not different between the two diabetes groups ($^{\dagger}p=0.099$). $^{\ddagger}p=0.003$ for HC vs MV−

Indeed, an inverse relationship between age and intra-epidermal nerve fibre density has been previously described [18, 19]; the influence of sex, height, weight and BMI is less certain [18, 20, 21].

It is also known that duration of diabetes and cardiovascular risk factors are associated with the development of clinical large fibre neuropathy [17, 22]. It is conceivable that these could be similarly associated with small fibre dysfunction. We do not believe that any of these factors could explain our findings as one of the strengths of this study is its case-controlled design, which allows for these factors. That we found no relationship between C-fibre function and duration of diabetes is not surprising as our study was not designed to specifically investigate this, individuals in both groups having been selected and matched for having moderate to long duration of diabetes.

The finding of higher triacylglycerol levels in the MV+ group compared with the MV− group is of interest as recent studies have reported an association between triacylglycerol levels and the presence of neuropathy [23, 24]. None of the patients in this study was receiving fibrate treatment. Though of interest, the relevance of this finding is uncertain as the difference was only just significant and there was no correlation with LDIflare size.

The scanned image from which the LDIflare is derived depends on the presence of intact C-fibres as well as the ability of the microvasculature to sufficiently dilate in order for detection by the LDI. Thus, the reduced LDIflare in the MV+ patients could theoretically reflect severely impaired vasodilatory capacity due to microvascular disease, rather than small fibre dysfunction. However, all individuals studied were able to vasodilate to the extent that they could increase microvascular blood flow to above 300 PU. They would, therefore, have had detectable LDIflares even if their maximum

vasodilatory capacity was reduced. Furthermore, although LDImax was similarly reduced in the MV− and MV+ groups, the LDIflare in the MV− group was greater and not significantly different from that in the control group; this would not expected if LDIflare was related to LDImax. Finally, as with our previous studies, there was no correlation between LDImax and LDIflare, confirming that they measure different physiological variables and are not influenced by each other within the range of microvascular blood flow studied [3, 9, 10]. LDImax represents the maximum vasodilatory capacity. Its reduction does not necessarily imply that microvascular blood flow will be insufficient to maintain normal tissue nutrition and hence normal neural function. Structural microangiopathy, however, is more likely to impact on tissue nutrition because of its multiple effects, including altered microvascular density, diffusion distances, vascular conductance and permeability. This could explain why a 20% reduction in LDImax was without any effect on small fibre function in those without evidence of structural microvascular disease in contrast to the impact in those with clinical microvascular disease.

Having considered and accounted for the previously described factors, there appears to be a strong association between C-fibre dysfunction and microangiopathy. In addition to the careful matching, another strength of this study was to have documentation of $HbA_{1c}$ measures in every diabetic individual for the entire duration of their diabetes. This revealed that there is also a strong relationship between C-fibre function and diabetes control, as represented by duration-averaged $HbA_{1c}$. Indeed, this relationship persisted when adjusted for age, height, weight and triacylglycerol. Had we just used the current $HbA_{1c}$ and blood glucose at the time of the study, this relationship would not have been apparent and we may have concluded that there was no relationship with glycaemic control. Whether the association between C-fibre dysfunction and microvascular disease is causal or whether both are the result of a greater glycaemic burden and are independent of each other will require further study. In this context, it is of interest that a recent report from the European Diabetes (EURODIAB) Prospective Complications Study Group concluded that microvascular complications strongly impacted on large fibre function as represented by nerve conduction velocity and amplitude [25]. The authors suggested there to be a common pathophysiological pathway of microvessel disease related to duration of diabetes and overall diabetes control. The present study suggests a similar pathophysiological pathway may occur for small fibre neuropathy.

The results of this study contrast with our previous finding of impaired C-fibre function in individuals with IGT who were free from microvascular disease and would have had a considerably lower glycaemic burden [9]. This disparity suggests possible differences in the aetiopathogenesis of small fibre dysfunction in the two forms of diabetes. Thus, microangiopathy resulting in nerve ischaemia and impaired nutrition may account for the neuropathy in type 1 diabetes, whereas altered neural cellular metabolism linked to the metabolic syndrome or abnormal insulin action may be implicated in IGT and early type 2 diabetes [26]. The importance of microangiopathy in the pathogenesis of neuropathy has already been well established for large fibres by the studies of Malik and others [27, 28]. Malik et al. demonstrated a relationship between the structure and function of large fibres and the presence of endo- and perineurial microangiopathy [28]. Whether two different aetiopathogenic mechanisms exist for small fibre neuropathy in the two types of diabetes suggested by our findings will, however, require further study. It is of course possible and likely that for many individuals both mechanisms may co-exist, especially in type 2 diabetes in which early-onset C-fibre dysfunction related to the metabolic syndrome may be worsened by the later development of neural microangiopathy. One of the strengths of our study was the careful exclusion of people with features of the metabolic syndrome from all the study groups to remove this as a potential confounder.

A potential limitation of this study is its reliance on a single measure of small fibre function. We did not include measurements of skin nerve fibre density as we had already demonstrated a good correlation with intra-epidermal nerve fibre density (IENFD) and dermal nerve fibre density. Another potential limitation is the fact that the assessor was not blinded. However, the LDIflare gives objective data of C-fibre function captured on a computer. It is important to point out that in order to explore the hypothesis only individuals free from clinical neuropathy could be included. As the participants had had diabetes for an average of 18 years, it might be considered that this is an unusual and unrepresentative cohort; however, this is not the case. The Epidemiology of Diabetes Interventions and Complications (EDIC) study at 13–14 years after study closure (average diabetes duration of 26 years) found normal nerve conduction studies in 46% and 31% and clinical neuropathy in only 34% and 41% of intensively treated and conventionally treated patients, respectively [29].

In summary, we have shown that in patients with type 1 diabetes the LDIflare test detects small fibre dysfunction, even when clinical neuropathy is absent, in individuals with microvascular disease. Furthermore, both small fibre dysfunction and microvascular disease are related to glycaemic burden as represented by duration-averaged $HbA_{1c}$. Despite their long duration of diabetes, individuals without microvascular disease had normal small fibre function. These findings underscore the importance of maintaining good long-term glycaemic control in type 1 diabetes.

Although the results support the hypothesis that different pathogenic mechanisms may be involved in the aetiology of

small fibre neuropathy in type 1 diabetes compared with that of the metabolic syndrome, a longitudinal study would be required to confirm this. Ideally, this should include corneal confocal microscopy and IENFD as measures of small fibre structure. If confirmed, this could have implications when investigating new therapies. For example, therapies aimed at putative metabolic defects may need to exclude those with established microvascular complications and type 1 diabetes and, similarly, investigations of therapies aimed at improving microvascular function may be best limited to those with type 1 diabetes without the features of the metabolic syndrome.

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**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

**Contribution statement** PV and GR recruited subjects, performed the procedures, analysed data and wrote the manuscript. AG contributed to technique development, study design and data analysis, and edited the manuscript. All the authors gave final approval of the version to be published.

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