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Letters to the Editor

Replication studies of *MIR149* association in Charcot–Marie–Tooth disease type 1A in a European population

A recent publication in your journal reported the identification of a genetic modifier, microRNA149 (*MIR149*), of Charcot–Marie–Tooth disease type 1A (CMT1A) [1]. In a sample of Korean patients, Nam et al. demonstrated significant association of the common SNP rs2292832 in *MIR149* with age at onset (AAO) and severity of CMT1A. They also noted that the varying minor allele frequency (MAF) of rs2292832 in ancestral groups: the frequency of the C allele is 0.363 in East Asians, 0.718 in Europeans, and 0.728 in Africans [2]. Therefore, the authors suggested that the association of rs2292832 might be ancestry specific. To complement and potentially replicate these findings, we are reporting association analysis results at this genomic locus for the CMT1A cohort of the Inherited Neuropathy Consortium. We studied 644 CMT1A patients collected over the past 9 years under standardized clinical research protocols. Principal component analysis confirmed European ancestry in all patients. We also performed copy number variation analysis confirming that all patients carry the canonical 1.5-Mb duplication on chromosome 17, which causes CMT1A.

In replicating the Nam et al study, we analyzed three clinical outcomes in CMT1A: self-reported AAO when patients first showed difficulty walking (226 patients available), CMT neuropathy score (CMTNS) (367 patients available), and median motor nerve conduction velocity (MNCV) (229 patients available). To avoid bias caused by relatedness in patients, only unrelated individuals were included in the analysis for each phenotype. Our phenotype analysis did not show significant difference in disease onset or severity between males and females. CMTNS increases with age (0.188 for every additional year of age, $P < 2 \times 10^{-16}$), consistent with a previous report [3].

We performed initial association analyses of all three phenotypes using linear regression. Two genetic models were used to assume the allelic effect of the minor allele T of rs2292832 (CC = 0, CT = 1, TT = 2 in the additive model, CC = 0, CT = TT = 1 in the dominant model). Patients' age at exam was included as a covariate in the analysis of CMTNS. However, we did not identify any significant association of rs2292832 with the three phenotypes. In the additive model, the *P* values were 0.1061 for AAO, 0.2444 for CMTNS, 0.3180 for MNCV. In the dominant model, the *P* values for three phenotypes were 0.1986, 0.5099 and 0.8878, respectively.

We then stratified the samples into early-onset/late-onset groups, and mild/severe groups, and tested the phenotypes as binary outcomes. We have 141 patients with early disease onset (AAO < 20 years), and 85 patients with late disease onset (AAO ≥ 20 years). Chi-square test did not identify any significant association ($P = 0.2580$, OR = 1.271, 95% CI of OR: 0.8386–1.926). We then analyzed CMTNS as a binary phenotype (274 individuals with CMTNS > 10 in the severe group, and 93 individuals with CMTNS ≤ 10 in the mild group). The analysis was performed using logistic regression, including patients' age at exam as a covariate. The result was also negative ($P = 0.6884$, OR = 0.929, 95% CI: 0.6481–1.332 in additive model. $P = 0.8285$, OR = 1.058, 95% CI: 0.6362–1.759 in dominant model). We have 102 severe patients with MNCV ≤ 20, and 127 mild patients with MNCV > 20. Using chi-square test, the association was not significant either ($P = 0.7593$, OR = 0.9382, 95% CI: 0.6238–1.411).

Finally, we expanded our analyses to 30 SNPs surrounding the *MIR149* locus within ±50 kb of rs2292832. Using a Bonferroni threshold of $P = 0.00161$, we did not identify any SNP associated with the three phenotypes. SNP rs3828334 (chr2:241390002, located 5 kb upstream of *MIR149*) showed *P* value of 0.0297 in association with CMTNS, which would be significant under the assumption of a single locus testing.

In conclusion, our evidence did not support *MIR149* as a genetic modifier of CMT1A in an independent European cohort. This may not necessarily rule out *MIR149* as a genetic modifier as our sample was relatively small for an association study and the effect may be ancestry specific, as pointed out by Nam et al. The small sample sizes in rare diseases are a major challenge for statistical approaches, and may only capture the largest effects sizes. A stable replication signal would require a much larger sample size as shown by GWAS studies in common disease [4]. We thus think that additional

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functional or family-based studies will be required to further validate the role of *MIR149* in CMT1A.

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Replication studies of *MIR149* association in Charcot–Marie–Tooth disease type 1A in a European population - response

We would like to thank Dr. Züchner and his colleague for giving us valuable comments about our recently published paper [1,2]. We agree with their conclusion that the rs2292832 single nucleotide polymorphism (SNP) of *MIR149* (n.86T > C) has no significant association between phenotypic heterogeneity and genotypes in the Charcot-Marie-Tooth disease type 1A (CMT1A) patients with European ancestry [2]. We previously performed an association study between the rs2292832 polymorphism and the clinical phenotypes of CMT1A in 176 unrelated Korean CMT1A patients [1]. From the study, we suggested that the TC and CC genotypes of the rs2292832 were significantly associated with the late onset and mild symptoms of CMT1A. Thus, we suggested that the rs2292832 variant in the *MIR149* may be a potential candidate for the genetic modifiers affecting the phenotypic heterogeneity of CMT1A.

It is known that CMT1A is uniformly developed due to a 1.5-fold increased dosage of *PMP22*; however, a wide range of phenotypic variations has been frequently noted [3]. Because miRNAs have an important role in the regulation of gene expression, we expected that variants of the miRNAs might be associated with the phenotypic heterogeneity of CMT1A. This study noticed a common single nucleotide polymorphism (n.86T > C, rs2292832) in *MIR149* which was predicted to target several CMT-related genes including *PMP22*. The rs2292832 was located near the 3′ end of the precursor miRNA of *MIR149* (Suppl. Fig. 1A).

Initially, *MIR149* was not the only target of our research. We searched for *MIR* gene SNPs that have minor allele frequencies (MAF) > 0.1 and are likely to target CMT-related genes including *PMP22* through several miR-target gene prediction programs including TargetScanHuman (http://www.targetscan.org/), DIANA-microT (http://diana.imis.athena-innovation.gr/), RNA22 (https://cm.jefferson.edu/rna22), MirTarget2 (http://mirdb.org/), and miRanda (http://www.microrna.org). Among the filtered results, *MIR149* was noticed. The rs2292832 SNP is located near the 3′ end of the precursor which is an important region for maturation (Suppl. Fig. 1a). *MIR149* was also predicted to interact with many CMT genes including *PMP22*, *SH3TC2*, *MPZ,* and *LITAF* (Suppl. Fig. 1b). For these reasons, we chose this SNP as a candidate and observed a significant association. Interestingly, this SNP has been previously published in several papers that it could cause a structural change and may alter gene expression [4,5].

MIR149 also contains another SNP, n.83A > G (rs71428439) at the adjacent sequence of rs2292832 (Suppl. Fig. 1a). Although this SNP was not analyzed in the previous study, a subsequent study showed that it also had a significant association between the genotypes and clinical phenotypes of CMT1A in the same samples [1]. The genotype frequencies of this SNP was significantly deviated from the Hardy-Weinberg equilibrium (HWE) ($p = 0.018$) in the patients group (Suppl. Table 1). It showed significantly different genotype distributions between the early onset (<20) and late onset (≥20) groups in the dominant model (Suppl. Table 2). The AG and GG genotypes were also significantly associated with late onset and/or mild clinical phenotypes (Suppl. Table 3). Haplotype analysis showed that the two SNPs were strongly linked, and the G-C haplotype was significantly associated with late onset compared to the A-T haplotype (Suppl. Table 4).

The MAFs of rs71428439 are not so different among ethnic groups (Korean: 0.179, Europeans: 0.127, and Africans: 0.112), while the MAFs of rs2292832 are very different, as mentioned in the previous report [1]. Thus, it is also necessary to consider that the combination of two SNPs may be an important factor. Recently, Sinkiewicz-Darol et al. suggested that p.I92V in *LITAF* is associated with an earlier onset of CMT1A and hereditary neuropathy with pressure palsies (HNPP), and it may have a role as a genetic modifier for clinical heterogeneity [6]. We also wanted to examine the role of p.I92V in Korean patients, but the allele frequency was too low to perform an association study. The ethnic group-specific allele frequencies

of these SNPs may relate to different roles according to ethnicity.

As mentioned by Tao and Züchner [2], the small sample sizes in rare diseases are a major challenge for statistical approaches. Therefore, to further validate the role of *MIR149* SNPs in CMT1A, large-scale association studies such as GWAS should be done in subjects with different ethnic groups.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2018.12.013.

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