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# *NET* gene silencing by *let-7i* in postural tachycardia syndrome

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**Introduction & Aim:** While strongly implicated in Postural Tachycardia Syndrome (POTS) and essential hypertension, considerable controversy exists regarding norepinephrine transporter (*NET*) loss-of-function. Increased heart rate in POTS is a malfunction of autonomic nervous system characterized by clinical symptoms of orthostatic intolerance, light-headedness, fatigue and near syncope on upright posture. *NET* is encoded by *SLC6A2* (*NET*) gene. Current evidence suggests that *NET* is regulated by methyl CpG binding protein 2 (MeCP2). Here *let-7i* directed binding of MeCP2 at *NET* promoter was investigated. Furthermore, it was tested whether pharmacological histone deacetylase inhibition (HDACi) could restore *NET* expression.

**Methods:** We developed a novel technique to identify RNAs bound to chromatin at *NET* prompter. Identification of chromatin associated RNAs at a specific locus can shed light on role of specific RNA in gene regulation affecting chromatin structure. Patients with POTS with the common clinical characteristics were recruited. Peripheral blood mononuclear cells (PBMCs) from POTS individuals were subjected to RNA immunoprecipitation sequencing (RIP-Seq), a novel RNA of isolated chromatin sequencing (RICh-Seq) and *ex vivo* stimulation by pharmacological HDAC inhibitor Suberoylanilide hydroxamic acid (SAHA (2 μM)).

**Results:** We show that *let-7i* is associated with *NET* suppression in POTS using unbiased RICh-Seq. *Let-7i* expression was higher in POTS as compared to healthy individuals. Increased *let-7i* binding at *NET* promoter and with MeCP2 in POTS subjects suggest that MeCP2 recruitment to chromatin is directed by *let-7i*. *Ex vivo* HDACi in PBMCs restored normal *NET* expression. Furthermore, *let-7i* inhibition in combination with HDACi alleviated transcriptional inhibition of *NET*.

**Conclusion:** MeCP2 recruitment to *SLC6A2* promoter is directed by *let-7i*. *NET* gene expression is restored by pharmacological HDAC inhibition suggesting this class of drugs may be useful therapeutics for POTS patients..

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