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Supplementary information

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Pre-symptomatic Parkinson's disease blood test quantifying repetitive sequence motifs in transfer RNA fragments

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SUPPLEMENTARY INFORMATION

Supplemental information for Extended Data Figure 2

To test if RGTTCRA-tRFs behave as a group with a characteristic expression pattern, we ran a binomial test, and found that the levels of 41% of all tRFs were reduced in PD CSF. In contrast, 53% of the RGTTCRA-tRFs were elevated ($p<2.3x10^{-6}$, FDR), and 96% of the MT-tRFs were reduced in PD ($p<1.6x10^{-13}$, FDR), whilst 38% of the N-tRFs lacking the RGTTCRA motif were elevated in PD ($p<0.09$, FDR). Furthermore, a Chi square test showed that the numbers of PD-elevated RGTTCRA-tRFs were 28% greater than what would be expected by random distribution ($p<6x10^{-10}$, Chi-square test), also when testing only tRF families which gave rise to RGTTCRA-tRFs (i.e., i/3'-N-tRFs; $p<1x10^{-10}$, Chi-square test) and when analyzing each sex separately (Extended Data Figure 2F, G).

Supplemental information for Extended Data Figure 3

We analyzed the GSE130764 dataset of Ang overexpression (OE; in HEK293T cells) and knockout (KO; in U2OS cells). The latter tested with and without sodium arsenite-induced oxidative stress.

Supplemental information – Blood tRF levels creates a patient-specific fingerprint

To challenge the ability of blood-tRFs to successfully constitute a patient-specific "fingerprint" we tested blood tRFs in all PPMI patients with 4 timepoints of blood withdrawal (n=1272 samples from 318 subjects). Our analysis revealed that tRNA-derived fragments indeed provide a stable and distinguishable profile for individuals over time. Among the classifiers tested, the Logistic Regression model demonstrated the highest performance, achieving a Matthews Correlation Coefficient (MCC) of 0.7483 before tuning and 0.7608 after hyperparameter optimization. This performance significantly outpaced other classifiers, with the next best being Linear SVC (MCC: 0.4361) and XGBoost (MCC: 0.3381). The stark contrast between the tuned Logistic Regression model's performance (MCC: 0.7608) and that of a dummy classifier trained on shuffled labels (MCC: -0.00316057) further underscores the presence of a consistent, individual-specific signal in the tRF profiles. These results support our hypothesis that tRFs form a stable, identifiable fingerprint for each individual.

Supplemental information - motif-carrying tRFs with ribosomes

To seek interactions of the motif-carrying tRFs with ribosomes by an independent approach, we analyzed the GSE113751 dataset of short RNA-seq of pulled-down ribosomes from the HeLa, HCT116 and HEK293T cell lines, before and after 3- or 6-hours exposure to Arginine or Leucine starvation. Intriguingly, in both healthy and starved cells RGTTCRA-tRFs consisted of 50-70% of the total tRF reads, demonstrating pronounced enrichment of motif-carrying tRFs compared to their share in tRF subtypes (~38% of all tRFs carry the motif; p values for all but one of the different samples ranged from 5e-6 to 2e-43, chi square test, FDR). The enrichment of ribosomal bound motif-carrying tRFs was consistent even when comparing the share of motif-containing tRFs to the random (50%) chance (p values of all samples with over 15 reads ranged from 0.014 to 1e-10, chi square test, FDR).

Supplemental information for Extended Data Figure 6

Importantly, RGTTCRA-tRFs mainly originate from the 3'-tRF and i-tRF subtypes. Therefore, we further sought specific changes in these two tRF subtypes prior to, right after or two hours post-depolarization. RGTTCRA-3'-tRFs and i-tRFs presented identical ribosomal enrichment and 2h pDP decline, whereas motif-lacking i/3'-tRFs showed less significant enrichment in ribosomes compared to cytoplasmic profiling (~1.5 fold higher, $p<3e-6$, ANOVA). Further, ribosomal profiling of motif-lacking i/3'-tRFs remained unchanged in the 2h pDP condition.