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LETTER TO EDITOR

Oxygen consumption rate for evaluation of *COQ2* variants associated with multiple system atrophy

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To the editor:
We identified homozygous or compound heterozygous mutations in *COQ2* in multiplex families with multiple system atrophy (MSA) and found that functionally impaired *COQ2* variants are associated with sporadic MSA [1]. In particular, association of V393A variant with risk of sporadic MSA has been confirmed in East Asians by a recent meta-analysis [2]. *COQ2* encodes parahydroxybenzoate-polyprenyl transferase, which is essential for the biosynthesis of coenzyme Q_{10} (CoQ_{10}) that serves as an electron carrier in the mitochondria.

Although the COQ2 activities of lymphoblastoid cell lines (LCLs) with heterozygous V393A were mildly but significantly decreased, the growth rate of the yeast strain harboring V393A *COQ2* cDNA was not [1]. Since the availability of LCLs is limited, sensitive assay methods for *COQ2* variants are important. Here, we measured the basal oxygen consumption rate (OCR) of the yeast *coq2* null strain harboring wild-type or mutant human *COQ2* (*hCOQ2*) cDNAs to evaluate the functional changes of COQ2.

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hCOQ2 cDNA was prepared from human placenta RNA by RT-PCR. The cloned cDNA spans 1266 bp including the first ATG codon (NM_015697.7). Mutant *hCOQ2* cDNAs (V393A [1–3], S146N [4], and M128V [1, 3]) were generated by site-directed mutagenesis followed by subcloning into pAUR123 (Takara Bio, Shiga, Japan). The BY4741Δ*coq2* strain was transformed with pAUR123 carrying wild-type or mutant *hCOQ2* cDNAs.

Basal OCR was measured at 30 °C using an XFe24 Extracellular Flux Analyzer (Seahorse Bioscience, North Billerica, MA) according to the manufacturer's instructions. Yeast cells were resuspended in 600 μL of non-fermentable glycerol medium and seeded onto the XF24 Cell Culture Microplates precoated with Poly-$_L$ lysine (Wako, Osaka, Japan) followed by centrifugation at 50×*g* for 1 min. The linearity of the basal OCR measurement was evaluated by measuring the basal OCRs of various numbers of yeast cells (0.5×10^5 to 4.0×10^5 cells) transformed with wild-type *hCOQ2*. Next, the mean basal OCRs of 1.0×10^5 yeasts with wild-type and mutant *hCOQ2* were compared using two-tailed *t* test with Bonferroni's correction. *P* values less than 0.05 were considered to indicate a statistical significance.

The linearity of the OCR measurement was confirmed in the range of 0.5×10^5 to 4.0×10^5 cells/well ($R^2 = 0.9998$; Fig. 1a). The mean basal OCRs of wild-type, V393A, S146N, and M128V were determined to be 148.7 ± 12.6, 103.4 ± 17.6, 75.7 ± 14.6, and 98.7 ± 24.7 pmol/min, respectively (Fig. 1b; mean ± SD).

The mean basal OCRs of the three mutants were significantly lower than that of the wild type, demonstrating that the three mutants, including V393A, were functionally impaired. Notably, the decrease in the basal OCR observed for S146N was more marked than those for V393A and M128V. Homozygous S146N was described in primary CoQ_{10} deficiency with a severe phenotype [4], whereas homozygous V393A was observed in MSA patients exhibiting much milder clinical

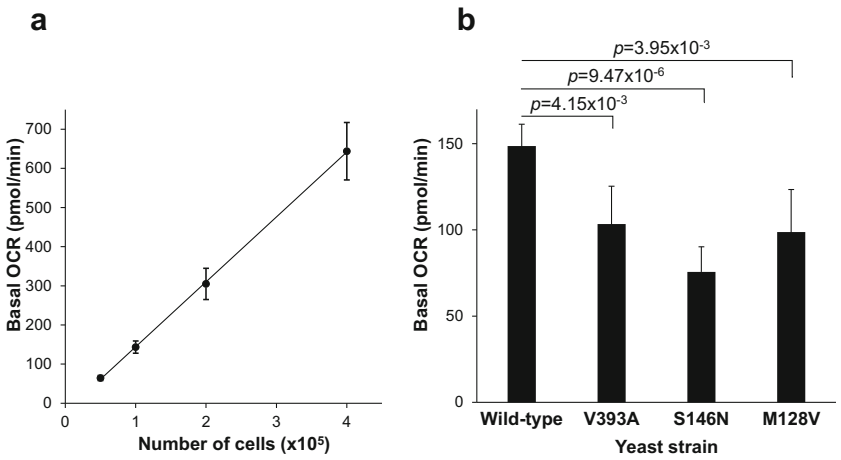

Fig. 1 Measurement of mean basal OCRs. **a** The mean basal OCRs of various numbers of yeasts transformed with wild-type *hCOQ2* cDNA were determined by three independent measurements. Error bars represent standard deviations. **b** The mean basal OCRs of yeasts transformed with wild-type or mutant *hCOQ2* cDNAs (V393A, S146N, and M128V) were determined by six independent measurements. The mean basal OCRs of yeast strains harboring mutant *hCOQ2* cDNAs were significantly lower than that of the yeast strain harboring wild-type *hCOQ2* cDNA. Error bars represent standard deviations

phenotypes [1]. The observation that the mean basal OCR observed for S146N was more markedly decreased suggests a correlation with the clinical severity. The present study indicates that measurement of basal OCRs of yeasts transformed with mutated *hCOQ2* cDNAs is useful for evaluation of the functional effects of *COQ2* mutations.

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