Long term exposure to cigarette smoke impacts genes involved in cardiac muscle structure and function in Apoe-/- mouse while exposure to a candidate modified risk tobacco product, the Tobacco Heating System 2.2 (THS 2.2) aerosol does not.

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Introduction
Heart failure is reported to affect over 15 million people in Europe (1) and over 5 million people in the United States (2), and is a major cause of hospitalization and morbidity (3). Smoking is a major cause of the high incidence of cardiovascular disease (4-5) and is highly associated with endothelial dysfunction, atherosclerosis and heart failure. Although heart failure is a serious complication of atherosclerosis, other stressors such as diabetes, hypertension, and toxic compounds can impact cardiac function and favor the development of cardiomyopathy, eventually causing heart failure. Candidate modified risk tobacco products have been developed to reduce the harm of smoking linked to cardiovascular and other smoking-related diseases. One such product, the Tobacco Heating System 2.2 (THS 2.2), which heats the tobacco instead of burning it, was developed to decrease significantly the levels of harmful and potentially harmful constituent levels, such as aldehydes and polycyclic aromatic hydrocarbons (5). To investigate the relative impact of exposure to an aerosol from THS2.2 compared with smoke from the 3R4F reference cigarette, as well as the impact of cessation or switching to THS2.2 after 2 months exposure to 3R4F smoke, we have conducted an inhalation study on Apoe-/- mice with a number of toxicological and disease-related endpoints (5).

Because there is substantial evidence that smoking is one of the risk factors of the development of cardiovascular pathologies, we also analyzed heart tissue of Apoe-/- mice from this study. A transcriptomics approach was chosen to identify at least some of the molecular mechanisms underlying the biological effects of exposure to 3R4F smoke and THS2.2 aerosol on the heart.

Study design and methods

### Study design

- **Apoe-/- mice**, (age 8-10 weeks) were exposed to 3R4F smoke or to THS2.2 aerosol for up to 8 months.
- Diluted mainstream smoke extracted from 3R4F cigarettes (600 mg total particulate material/3mL, equivalent to 29.0 mg nicotine/3mL) THS2.2 aerosol (nicotine-matched to 3R4F, 29.9 mg/ml), or filtered air where used to expose mice (whole body exposure), during 3 hours per day, 5 days per week, for up to 8 months. To avoid a bulk of excessive carbonylhydroxyl containing in the THS2.2 group, intermittent daily exposure to fresh filtered air for 30 min after the first hour of smoke exposure and for 60 min after the third hour of exposure. At the end of each month, Apoe-/- mice exposed to fresh air ( sham) were challenged with a control group. After two months of exposure to 3R4F smoke, subsets of mice were exposed to fresh air to mimic smoking cessation or were switched to THS2.2 (6).

### Transcripts analysis

- **One month after exposure**, only six genes were significantly deregulated (FDR <0.05). Glcrcl, Car14, Gm5793, and Sncra were upregulated, and Ifh14, Ide1, Collaf1, and Pomt were downregulated. After 2 months of 3R4F smoke exposure, only two genes were significantly impacted: Glcrcl was upregulated, and Rbp7 was downregulated. After 3 months of 3R4F smoke exposure, 44 genes were significantly differentially expressed in response to 3R4F exposure. After 6 months, the number of differentially expressed genes was considerably higher, and this number remained relatively stable at 8 months of smoke exposure. In the heart tissues from mice exposed to THS2.2 aerosol or those switched to fresh air or THS2.2 after 2 months of 3R4F smoke exposure, no differentially expressed genes were detected compared with the sham groups at all time points evaluated.

#### Gene set enrichment analysis (GSEA) (**B**)

The GSEA showed significant downregulation of gene sets involved in muscle structure and function, affection the actin cytoskeleton, focal adhesion sites, gap junctions, and adherent junctions in the hearts of 3R4F smoke exposed mice. No such downregulation was detected for the same gene sets in the THS2.2, cessation, and switching groups.

The GSEA analysis predicted a significant downregulation of inflammatory pathways associated with "NF-Kappa B signaling", "Chemokine signaling", "TNF signaling", "Leucocyte transendothelial migration", "Filamin signaling", and "Cytokine-cytokine receptor interactions". 3R4F smoke exposure additionally involved genes in "Drug metabolism-cytocchrome P450" and "Metabolism of xenobiotics by cytochrome P450" and downregulated genes involved in ECM-receptor interaction.

Differential gene regulation in the hearts of mice in the switching groups was observed. At the 8-month time point, the gene set related to fatty acid elongation was downregulated in the hearts of mice in the switching group; however, the regulation was at a notably lower level than in mice continuously exposed to 3R4F smoke. The regulation of gene sets related to amino sugar and nucleotide sugar metabolism, as well as with citrin transport and bile acid transport was up-regulated in the switching group at the 3 and 6-month time points compared with the THS2.2 group at the same time points.

### References