

Supplemental Material

Using Residual Newborn Blood Spots to Investigate CpG Methylation in Relation to Air Pollution and Congenital Heart Defects

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S1. DNA Methylation Quality Control (QC) Pipeline

This document details the DNA methylation quality control (QC) pipeline that has been implemented by the Fry Laboratory (Clark et al. 2022; Eaves et al. 2023; Santos et al. 2023).

The steps in the DNA methylation QC pipeline include: (1) loading IDAT files; (2) visually inspecting DNA methylation data with ShinyMethyl; (3) removing sex mismatches with a sample filter; (4) application of a probe filter; (5) utilizing a normalization function; (6) identifying and removing batch effects; and (7) generating final beta values. These steps are depicted in Figure S1 and described in the text below.

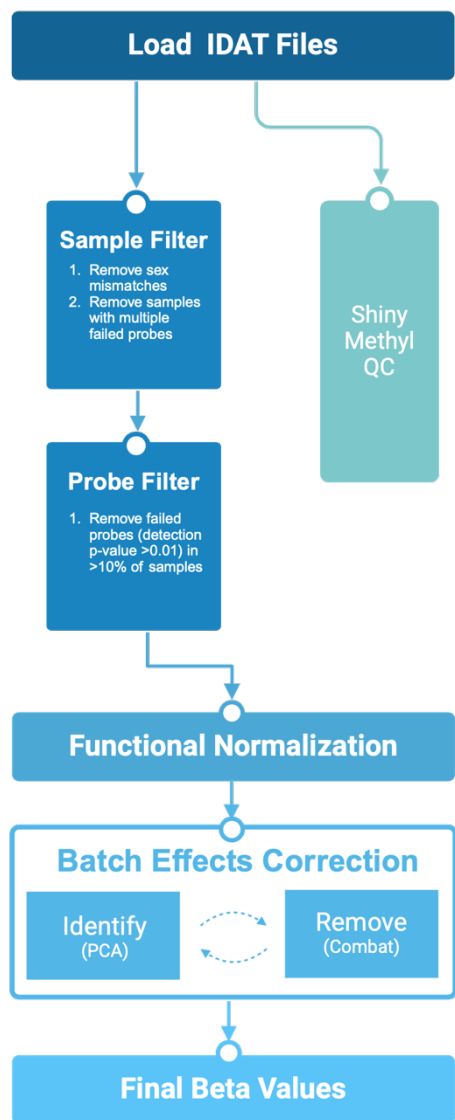


Figure S1: Visual depiction of the steps in the QC pipeline.

1. Load IDAT Files

The first step is to load in the raw data, IDAT files. Each sample has two files, one for the red channel and one for the green channel. This data is loaded into an RGChannelSet object using minfi in R.

2. ShinyMethyl

ShinyMethyl is an R package designed to visually inspect DNA methylation data. This is used on the raw data, primarily, and is used to visually inspect the data. It can be used to assess outliers, sex mismatches, etc. In this pipeline, this is done simultaneously or prior to the sample and probe filters. Samples that were not removed or caught using the sample filter checks, may be further looked at and potentially removed.

3. Sample Filter

The sample filter is used to remove samples that are considered sex mismatches or have many failed probes. Sex mismatches are determined using the getSex function in minfi. From the minfi documentation: “Estimation of sex is based on the median values of measurements on the X and Y chromosomes respectively. If yMed - xMed is less than cutoff we predict a female, otherwise male.”

Failed probes are identified using detection p-values, calculated using the detectionP function in minfi. This function “compares the total DNA signal (Methylated + Unmethylated) for each position to the background signal level. The background is estimated using negative control positions.” Values >.01 are considered failed probes.

4. Probe Filter

The probe filter is similar to the sample filter using detection p-values, except this is done across samples. If a probe has failed (i.e. has detection p-values > .01) for greater than 10% of the samples, this probe is removed from the dataset.

5. Normalization

This QC pipeline utilizes functional normalization, with a first step of noob background correction. The function used in minfi is preprocessFunnorm. From minfi, “Functional normalization (FunNorm) is a between-array normalization method for the Illumina Infinium HumanMethylation450 platform. It removes unwanted variation by regressing out variability explained by the control probes present on the array.” Though the documentation references 450 platform, it also works with EPIC (found in this article: *Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi*).

6. Batch Effects

Batch effects are identified using PCA in combination with visualizing data using different covariates. Typical ones looked at are position, chip, array, and date. If batch effects are found, ComBat is used to remove these effects.

7. Final Beta Values

Final beta values are generated from normalized (potentially ComBat adjusted) methylation data. Beta values are the ratio of the methylated probe intensity to the overall intensity (sum of methylated and unmethylated probe intensities). The range of beta values is between 0 and 1, where a beta value of 0 indicates that all copies of the CpG site in the sample were completely unmethylated. While a beta value of 1 indicates that every copy of the site was methylated.

75 **Table S1.** Significant canonical pathways by TOF status or air pollution exposure.

Ingenuity Canonical Pathways	-log (p-value)	non-log p-value	Ratio	Molecules	Gene set
Glutathione-mediated Detoxification	5.47	0.00000342	0.0789	<i>GSTM1, GSTM2, GSTM5</i>	TOF
Xenobiotic Metabolism AHR Signaling Pathway	4.38	0.0000419	0.0345	<i>GSTM1, GSTM2, GSTM5</i>	TOF
Xenobiotic Metabolism General Signaling Pathway	3.74	0.000183	0.021	<i>GSTM1, GSTM2, GSTM5</i>	TOF
Glutathione Redox Reactions I	3.66	0.000219	0.0714	<i>GSTM1, GSTM2</i>	TOF
Aryl Hydrocarbon Receptor Signaling	3.6	0.000251	0.0189	<i>GSTM1, GSTM2, GSTM5</i>	TOF
FXR/RXR Activation	3.39	0.000403	0.016	<i>GSTM1, GSTM2, GSTM5</i>	TOF
Xenobiotic Metabolism CAR Signaling Pathway	3.35	0.000449	0.0155	<i>GSTM1, GSTM2, GSTM5</i>	TOF
Xenobiotic Metabolism PXR Signaling Pathway	3.34	0.000456	0.0154	<i>GSTM1, GSTM2, GSTM5</i>	TOF
NRF2-mediated Oxidative Stress Response	3.1	0.000803	0.0127	<i>GSTM1, GSTM2, GSTM5</i>	TOF
LPS/IL-1 Mediated Inhibition of RXR Function	2.99	0.00102	0.0117	<i>GSTM1, GSTM2, GSTM5</i>	TOF
PXR/RXR Activation	2.93	0.00118	0.0308	<i>GSTM1, GSTM2</i>	TOF
Xenobiotic Metabolism Signaling	2.83	0.00149	0.0102	<i>GSTM1, GSTM2, GSTM5</i>	TOF
Apelin Adipocyte Signaling Pathway	2.64	0.0023	0.022	<i>GSTM1, GSTM2</i>	TOF
Class B/2 (Secretin family receptors)	2.59	0.00256	0.0208	<i>CALCA, CALCB</i>	TOF
G alpha (s) signalling events	2.24	0.00572	0.0138	<i>CALCA, CALCB</i>	TOF
Azathioprine ADME	1.99	0.0102	0.0769	<i>GSTM1</i>	TOF
Signal regulatory protein family interactions	1.9	0.0125	0.0625	<i>SKAP2</i>	TOF
Signaling by BMP	1.69	0.0203	0.0385	<i>SMAD9</i>	TOF
Cardiomyocyte Differentiation via BMP Receptors	1.68	0.021	0.037	<i>SMAD9</i>	TOF
Mitochondrial Dysfunction	1.53	0.0294	0.00581	<i>GSTM1, GSTM2</i>	TOF
Transcriptional Regulation by VENTX	1.51	0.031	0.025	<i>VENTX</i>	TOF
Carboxyterminal post-translational modifications of tubulin	1.46	0.0348	0.0222	<i>TTLL8</i>	TOF
Metabolism of non-coding RNA	1.37	0.0424	0.0182	<i>GEMIN6</i>	TOF
Primary Immunodeficiency Signaling	1.33	0.0469	0.0164	<i>AIRE</i>	TOF

Regulation of beta-cell development	1.86	0.0138	0.0238	<i>NR5A2</i>	PM2.5
Role of OCT4 in Mammalian Embryonic Stem Cell Pluripotency	1.82	0.0151	0.0217	<i>NR5A2</i>	PM2.5
Extracellular matrix organization	1.46	0.0346	0.00943	<i>TNR</i>	PM2.5
ESR-mediated signaling	1.42	0.0384	0.00847	<i>NR5A2</i>	PM2.5
Neuroinflammation Signaling Pathway	3.53	0.000296	0.0126	<i>CFLAR, HLA-DPA1, HLA-DPB1, PPP3CA</i>	Ozone
RIPK1-mediated regulated necrosis	3.3	0.0005	0.0625	<i>CFLAR, UBE2L3</i>	Ozone
Antigen Presentation Pathway	3.13	0.000744	0.0513	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
TNF signaling	2.8	0.00183	0.0351	<i>CFLAR, UBE2L3</i>	Ozone
Costimulation by the CD28 family	2.53	0.00294	0.0256	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
Methylmalonyl Pathway	2.38	0.00413	0.25	<i>MMUT</i>	Ozone
Interferon gamma signaling	2.37	0.00424	0.0213	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
2-oxobutanoate Degradation I	2.29	0.00516	0.2	<i>MMUT</i>	Ozone
PD-1, PD-L1 cancer immunotherapy pathway	2.26	0.00545	0.0187	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
MSP-ROn Signaling in Macrophages Pathway	2.17	0.0067	0.0168	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
Th1 Pathway	2.15	0.00703	0.0164	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
TCR signaling	2.13	0.00748	0.0159	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
MHC class II antigen presentation	2.13	0.00748	0.0159	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
TRAIL signaling	2.08	0.00825	0.125	<i>CFLAR</i>	Ozone
Th2 Pathway	2.06	0.00879	0.0146	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
Role of Chondrocytes in Rheumatoid Arthritis Signaling Pathway	2.03	0.00929	0.0142	<i>CFLAR, PPP3CA</i>	Ozone
IL-10 Signaling	1.96	0.011	0.013	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
Necroptosis Signaling Pathway	1.95	0.0111	0.0129	<i>CFLAR, PPP3CA</i>	Ozone
Calcium-induced T Lymphocyte Apoptosis	1.94	0.0115	0.00652	<i>HLA-DPA1, HLA-DPB1, PPP3CA</i>	Ozone
Th1 and Th2 Activation Pathway	1.87	0.0136	0.0116	<i>HLA-DPA1, HLA-DPB1</i>	Ozone
ICOS-ICOSL Signaling in T Helper Cells	1.82	0.015	0.00592	<i>HLA-DPA1, HLA-DPB1, PPP3CA</i>	Ozone

NUR77 Signaling in T Lymphocytes	1.81	0.0155	0.00585	<i>HLA-DPA1, HLA-DPBI, PPP3CA</i>	Ozone
CD28 Signaling in T Helper Cells	1.8	0.0158	0.0058	<i>HLA-DPA1, HLA-DPBI, PPP3CA</i>	Ozone
Macrophage Classical Activation Signaling Pathway	1.79	0.0162	0.0106	<i>HLA-DPA1, HLA-DPBI</i>	Ozone
Macrophage Alternative Activation Signaling Pathway	1.79	0.0164	0.0105	<i>HLA-DPA1, HLA-DPBI</i>	Ozone
Caspase activation via Death Receptors in the presence of ligand	1.78	0.0164	0.0625	<i>CFLAR</i>	Ozone
PKCθ Signaling in T Lymphocytes	1.71	0.0193	0.00538	<i>HLA-DPA1, HLA-DPBI, PPP3CA</i>	Ozone
Glucocorticoid Receptor Signaling	1.67	0.0216	0.00515	<i>HLA-DPA1, HLA-DPBI, PPP3CA</i>	Ozone
Calcium Signaling	1.67	0.0216	0.00909	<i>PPP3CA, TRPC3</i>	Ozone
Multiple Sclerosis Signaling Pathway	1.66	0.0219	0.00901	<i>HLA-DPA1, HLA-DPBI</i>	Ozone
Hepatic Cholestasis	1.66	0.0221	0.00897	<i>NR5A2, PPP3CA</i>	Ozone
Mitochondrial Fatty Acid Beta-Oxidation	1.61	0.0245	0.0417	<i>MMUT</i>	Ozone
T Cell Receptor Signaling	1.6	0.025	0.00487	<i>HLA-DPA1, HLA-DPBI, PPP3CA</i>	Ozone
Effects of PIP2 hydrolysis	1.56	0.0276	0.037	<i>TRPC3</i>	Ozone
Systemic Lupus Erythematosus in T Cell Signaling Pathway	1.55	0.0279	0.00467	<i>HLA-DPA1, HLA-DPBI, PPP3CA</i>	Ozone
Protein ubiquitination	1.51	0.0306	0.0333	<i>UBE2L3</i>	Ozone
Superpathway of Methionine Degradation	1.4	0.0396	0.0256	<i>MMUT</i>	Ozone
Regulation of beta-cell development	1.37	0.0426	0.0238	<i>NR5A2</i>	Ozone
Role of OCT4 in Mammalian Embryonic Stem Cell Pluripotency	1.33	0.0465	0.0217	<i>NR5A2</i>	Ozone
nNOS Signaling in Neurons	1.32	0.0475	0.0213	<i>PPP3CA</i>	Ozone
Nucleosome assembly	1.31	0.0495	0.0204	<i>CENPQ</i>	Ozone

76 TOF: Tetralogy of Fallot

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78 **Table S2.** Protein-protein interactions by TOF status or air pollution exposure.

Node 1	Node 2	Node 1 string id	Node 2 string id	Neighborhood on chromosome	Gene fusion	Phylogenetic cooccurrence	homology	Co-expression	Experimentally determined interaction	Database annotated	Automated text mining	Combined score	Gene set
<i>GSTM1</i>	<i>GSTM2</i>	9606.ENSP00000311469	9606.ENSP00000241337	0	0	0.05	0.981	0.999	0.695	0.65	0.058	0.999	TOF
<i>GSTM2</i>	<i>GSTM1</i>	9606.ENSP00000241337	9606.ENSP00000311469	0	0	0.05	0.981	0.999	0.695	0.65	0.058	0.999	TOF
<i>CALCA</i>	<i>CALCB</i>	9606.ENSP00000331746	9606.ENSP00000433490	0	0	0	0.938	0.671	0	0.9	0.256	0.973	TOF
<i>CALCB</i>	<i>CALCA</i>	9606.ENSP00000433490	9606.ENSP00000331746	0	0	0	0.938	0.671	0	0.9	0.256	0.973	TOF
<i>GSTM2</i>	<i>GSTM5</i>	9606.ENSP00000241337	9606.ENSP00000256593	0	0	0.05	0.98	0.137	0.847	0.65	0.059	0.951	TOF
<i>GSTM5</i>	<i>GSTM2</i>	9606.ENSP00000256593	9606.ENSP00000241337	0	0	0.05	0.98	0.137	0.847	0.65	0.059	0.951	TOF
<i>GSTM1</i>	<i>GSTM5</i>	9606.ENSP00000311469	9606.ENSP00000256593	0	0	0.049	0.982	0.102	0.61	0.65	0.056	0.87	TOF
<i>GSTM5</i>	<i>GSTM1</i>	9606.ENSP00000256593	9606.ENSP00000311469	0	0	0.049	0.982	0.102	0.61	0.65	0.056	0.87	TOF
<i>ASB3</i>	<i>GPR75</i>	9606.ENSP00000499751	9606.ENSP00000378195	0	0	0	0	0	0	0	0.646	0.646	TOF
<i>GPR75</i>	<i>ASB3</i>	9606.ENSP00000378195	9606.ENSP00000499751	0	0	0	0	0	0	0	0.646	0.646	TOF
<i>HLA-DPA1</i>	<i>HLA-DPB1</i>	9606.ENSP00000393566	9606.ENSP00000408146	0	0	0	0.674	0.808	0.932	0.9	0.93	0.999	Ozone
<i>HLA-DPB1</i>	<i>HLA-DPA1</i>	9606.ENSP00000408146	9606.ENSP00000393566	0	0	0	0.674	0.808	0.932	0.9	0.93	0.999	Ozone
<i>CFLAR</i>	<i>UBE2L3</i>	9606.ENSP00000312455	9606.ENSP00000400906	0	0	0	0	0.042	0.045	0.5	0.042	0.503	Ozone

<i>UBE2L3</i>	<i>CFLAR</i>	9606.ENSP0000 0400906	9606.ENSP0000 0312455	0	0	0	0	0.042	0.045	0.5	0.042	0.503	Ozone
<i>BOLL</i>	<i>SRSF8</i>	9606.ENSP0000 0314792	9606.ENSP0000 0480140	0	0	0.29	0	0	0.126	0	0.167	0.438	Ozone
<i>SRSF8</i>	<i>BOLL</i>	9606.ENSP0000 0480140	9606.ENSP0000 0314792	0	0	0.29	0	0	0.126	0	0.167	0.438	Ozone
<i>HLA-DPA1</i>	<i>SPATC1L</i>	9606.ENSP0000 0393566	9606.ENSP0000 0291672	0	0	0	0	0	0	0	0.425	0.425	Ozone
<i>SPATC1L</i>	<i>HLA-DPA1</i>	9606.ENSP0000 0291672	9606.ENSP0000 0393566	0	0	0	0	0	0	0	0.425	0.425	Ozone

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80 TOF: Tetralogy of Fallot

81 **Table S3.** Significant networks identified in Ingenuity Pathway Analysis (IPA) by TOF status or air pollution exposure.

Gene set	Molecules in Network	Score	Focus Molecules	Top Diseases and Functions
TOF	ACTA2, ADAMTSL2, APP, ASB3/GPR75-ASB3, AZ5104, CMTM2, CSNK2B, CTNNB1, CUL3, DLEU7, EGF:Ligand-responsive EGFR mutants dimer, EGFR, EGFRvIII mutant:HSP90:CDC37, EGFRvIII:GRB2:GAB1, EGFRvIII:GRB2:SOS1, EGFRvIII:PLCG1, EGFRvIII:SHC1, GEMIN6, HSP90AA1, Ligand-responsive EGFR mutant dimers, Ligand-responsive EGFR mutants:CBL, Ligand-responsive EGFR mutants:HSP90:CDC37, Ligand-responsive EGFR mutants:PLCG1, Ligand-responsive EGFR mutants:SHC1, LIM2, NAV1, NFkB (complex), PRPH, Sensitive ligand-responsive EGFR mutants:Non-covalent EGFR TKIs, SKAP2, VENTX, ZNF486, ZNF565, ZNF619, ZNF692	27	10	Cancer, Hair and Skin Development and Function, Organismal Injury and Abnormalities
TOF	12(S)-HPETE, 3, 4-epoxy-1-butene, 5-androstene-3, 17-dione, aflatoxin B1-2, 3-oxide, AIRE, alkyl halide, AW112010, beta-estradiol, CALCA, Calcb, CALCB, Cbp/p300/MAF/NFE2L2, CGRP1 receptor:CGRP, dimethyl maleate, epoxide, flavonoid, glutathione transferase, GPR75, GST, GST (complex), GST dimers, GSTM1, GSTM2, GSTM3, Gstm6l, Gstp3, Gsst3, GSTT4, LINC00992, Lncpint, RB1, Scgb1c1, Slpi/Slpil2, SMAD9, TNF	21	8	Cell-To-Cell Signaling and Interaction, Drug Metabolism, Nervous System Development and Function
TOF	acid-D-amino acid ligase, Carboxyterminal post-translational modifications of tubulin, TTLL3, TTLL8, TTLL10, TTLL8	3	1	Cell Morphology, Nervous System Development and Function, Tissue Morphology
PM2.5	12 lipoxxygenase, ADAMTS8, ADGB, AKR1E2, ANKRD30B, Ca2 , CALN1, CAPN11, CAPN5, CAPN9, CCDC152, Cmah, D-lactic acid, DCC dimer, EGFR ligand, fucose, IFNG, MMP9, MUC1, MYO1H, NIT2, NPS, NR5A2, PRKN, SCN (family), SIGLEC15, SLC24A4, sn-1, 2-didecanoylglycerol, ST18, Syndecan, TBC1D22A, TMEM176A, TNF, TRAM1L1, Wfdc18	25	8	Cellular Function and Maintenance, Cellular Growth and Proliferation, Hematological System Development and Function
Ozone	Activin A, AKT/Calmodulin/HSP90/NOS3, AR/androgen, CAK, Calbindin, Calmodulin, Calmodulin-HSP90-NOS3, Calmodulin/Ca2 , CAMK2N2, CDK16, CFLAR, CFLAR:LUBAC:Ub:UBE2D3, cNOS, FSH, FSIP1, Hsp90 (family), Insulin, MICB, miR-491-5p (and other miRNAs w/seed GUGGGGA), Nfat (family), Nfatc, NFkB (complex), NR5A2, PACRG, POLR2A, PPP3CA, RT1-EC2, SEC61, SLC3A1, THADA, TRPC, Trpc2, TRPC3, TRPM6, UBE2L3	25	10	Cell Signaling, Molecular Transport, Vitamin and Mineral Metabolism
Ozone	3830403N18Rik/Xlr, AGRN, Akt, AKT1, APOE, BOLL, butyryl-L-carnitine, CD74-NRG1, CLTC, CTLA4-CD28, Gm4836 (includes others), HLA-DPA1, HLA-DPB1, inositol 1-phosphate, inositol 2-phosphate, INSM2, KCNK15-AS1, miR-186-3p (miRNAs w/seed CCCAAAG), miR-718 (miRNAs w/seed UUCCGCC), MMP9, MMUT, N-arachidonoyl L-serine, PIN1-DT, PTCSC1, S-geranylgeranyl-L-glutathione, SLC6A15, SPATC1L, ST7-AS1, STAC, TBC1D22A, TMEM176A, TNF, TTR, UBE2L3-KRAS, WEE2-AS1	25	10	Cellular Assembly and Organization, Neurological Disease, Organismal Injury and Abnormalities
Ozone	APP, Atp5f1e, CALML4, CENPQ, CNDP1, ESR1, FAM133B, GFM2, GPR85, LINC-ROR, mir-191, miR-191-5p (and other miRNAs w/seed AACGGAA), miR-515-5p (and other miRNAs w/seed UCUCCAA), MPV17L, Mta, NENF, NSA2, PGLYRP2, POMZP3, PPP1R17, PUS7L, Rpl36, SLC5A7, SRSF8, SYCP2L, TMSB15A, TP53, TSKU, TSPY1 (includes others), Ttf1, TTLL6, UBXXN10, UFM1/UFL1, ZNF385B, ZNF839	9	4	Cell Death and Survival, Connective Tissue Disorders, Organismal Injury and Abnormalities
Ozone	GPI-anchored proteins, LYPD8	3	1	Digestive System Development and Function, Gastrointestinal Disease, Organismal Injury and Abnormalities

82 TOF: Tetralogy of Fallot; PM2.5: fine particulate matter

83 **Table S4.** Significantly enriched protein domains by tetralogy of Fallot (TOF) status or air pollution exposure.

Method	Term ID	Term description	Observed gene count	Background gene count	Strength	False discovery rate	Matching proteins in your network (IDs)	Matching proteins in your network (labels)	Gene set
SMART	SM00113	Calcitonin	2	3	2.84	0.0072	9606.ENSP00000331746, 9606.ENSP00000433490	<i>CALCA</i> , <i>CALCB</i>	TOF
InterPro	IPR036282	Glutathione S-transferase, C-terminal domain superfamily	3	38	1.91	0.0095	9606.ENSP00000241337, 9606.ENSP00000256593, 9606.ENSP00000311469	<i>GSTM2</i> , <i>GSTM5</i> , <i>GSTM1</i>	TOF
InterPro	IPR010987	Glutathione S-transferase, C-terminal-like	3	33	1.97	0.0076	9606.ENSP00000241337, 9606.ENSP00000256593, 9606.ENSP00000311469	<i>GSTM2</i> , <i>GSTM5</i> , <i>GSTM1</i>	TOF
InterPro	IPR040079	Glutathione transferase family	3	28	2.05	0.0062	9606.ENSP00000241337, 9606.ENSP00000256593, 9606.ENSP00000311469	<i>GSTM2</i> , <i>GSTM5</i> , <i>GSTM1</i>	TOF
InterPro	IPR004045	Glutathione S-transferase, N-terminal	3	25	2.09	0.0062	9606.ENSP00000241337, 9606.ENSP00000256593, 9606.ENSP00000311469	<i>GSTM2</i> , <i>GSTM5</i> , <i>GSTM1</i>	TOF
InterPro	IPR004046	Glutathione S-transferase, C-terminal	3	22	2.15	0.0062	9606.ENSP00000241337, 9606.ENSP00000256593, 9606.ENSP00000311469	<i>GSTM2</i> , <i>GSTM5</i> , <i>GSTM1</i>	TOF
InterPro	IPR021116	Calcitonin/adrenomedullin	2	5	2.62	0.0132	9606.ENSP00000331746, 9606.ENSP00000433490	<i>CALCA</i> , <i>CALCB</i>	TOF
InterPro	IPR003081	Glutathione S-transferase, Mu class	3	5	2.79	0.0003	9606.ENSP00000241337, 9606.ENSP00000256593, 9606.ENSP00000311469	<i>GSTM2</i> , <i>GSTM5</i> , <i>GSTM1</i>	TOF
InterPro	IPR001693	Calcitonin peptide-like	2	3	2.84	0.0095	9606.ENSP00000331746, 9606.ENSP00000433490	<i>CALCA</i> , <i>CALCB</i>	TOF
InterPro	IPR018360	Calcitonin, conserved site	2	3	2.84	0.0095	9606.ENSP00000331746, 9606.ENSP00000433490	<i>CALCA</i> , <i>CALCB</i>	TOF
InterPro	IPR021117	Calcitonin-like	2	3	2.84	0.0095	9606.ENSP00000331746, 9606.ENSP00000433490	<i>CALCA</i> , <i>CALCB</i>	TOF
Pfam	PF02798	Glutathione S-transferase, N-terminal domain	3	16	2.29	0.0017	9606.ENSP00000241337, 9606.ENSP00000256593, 9606.ENSP00000311469	<i>GSTM2</i> , <i>GSTM5</i> , <i>GSTM1</i>	TOF
Pfam	PF14497	Glutathione S-transferase, C-terminal domain	3	15	2.32	0.0017	9606.ENSP00000241337, 9606.ENSP00000256593, 9606.ENSP00000311469	<i>GSTM2</i> , <i>GSTM5</i> , <i>GSTM1</i>	TOF
SMART	SM00407	Immunoglobulin C-Type	3	40	1.77	0.0176	9606.ENSP00000252229, 9606.ENSP00000393566, 9606.ENSP00000408146	<i>MICB</i> , <i>HLA-DPA1</i> , <i>HLA-DPB1</i>	Ozone

84 **Table S5.** Genes with inferred connections to TOF, cardiovascular abnormalities, and/or congenital abnormalities by TOF status or air pollution exposure.

Gene	ID	Disease	Gene set
<i>AIRE</i>	326	Congenital abnormalities, cardiovascular abnormalities	TOF
<i>ASB3</i>	51130	Congenital abnormalities, cardiovascular abnormalities	TOF
<i>CALCB</i>	797	Congenital abnormalities, cardiovascular abnormalities	TOF
<i>PRPH</i>	5630	Congenital abnormalities, cardiovascular abnormalities	TOF
<i>TLL8</i>	164714	Congenital abnormalities, cardiovascular abnormalities	TOF
<i>ADAMTSL2</i>	9719	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>CALCA</i>	796	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>CMTM2</i>	146225	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>DLEU7</i>	220107	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>GEMIN6</i>	79833	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>GPR75</i>	10936	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>GSTM1</i>	2944	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>GSTM2</i>	2946	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>GSTM5</i>	2949	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>NAV1</i>	89796	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>SKAP2</i>	8935	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>SMAD9</i>	4093	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>VENTX</i>	27287	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>ZNF692</i>	55657	TOF, congenital abnormalities, cardiovascular abnormalities	TOF
<i>ADGB</i>	79747	TOF, congenital abnormalities, cardiovascular abnormalities	PM2.5
<i>AKR1E2</i>	83592	TOF, congenital abnormalities, cardiovascular abnormalities	PM2.5
<i>ANKRD30B</i>	374860	Congenital abnormalities, cardiovascular abnormalities	PM2.5
<i>NR5A2</i>	2494	TOF, congenital abnormalities, cardiovascular abnormalities	PM2.5
<i>TBC1D22A</i>	25771	TOF, congenital abnormalities, cardiovascular abnormalities	PM2.5
<i>TMEM176A</i>	55365	TOF, congenital abnormalities, cardiovascular abnormalities	PM2.5
<i>TNR</i>	7143	TOF, congenital abnormalities, cardiovascular abnormalities	PM2.5
<i>TRAM1L1</i>	133022	Congenital abnormalities, cardiovascular abnormalities	PM2.5
<i>BOLL</i>	66037	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>CDK16</i>	5127	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>CENPQ</i>	55166	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>CFLAR</i>	8837	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>FSIP1</i>	161835	Congenital abnormalities, cardiovascular abnormalities	Ozone

<i>GFM2</i>	84340	Congenital abnormalities, cardiovascular abnormalities	Ozone
<i>HLA-DPA1</i>	3113	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>HLA-DPB1</i>	3115	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>INSM2</i>	84684	Congenital abnormalities, cardiovascular abnormalities	Ozone
<i>LYPD8</i>	646627	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>MICB</i>	4277	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>MUT</i>	422049	Congenital abnormalities	Ozone
<i>NR5A2</i>	2494	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>NSA2</i>	10412	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>PACRG</i>	135138	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>PPP3CA</i>	5530	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>SLC6A15</i>	55117	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>SPATC1L</i>	84221	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>SRSF8</i>	10929	Congenital abnormalities, cardiovascular abnormalities	Ozone
<i>STAC</i>	6769	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>TBC1D22A</i>	25771	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>THADA</i>	63892	Congenital abnormalities, cardiovascular abnormalities	Ozone
<i>TMEM176A</i>	55365	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>TRPC3</i>	7222	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone
<i>UBE2L3</i>	7332	TOF, congenital abnormalities, cardiovascular abnormalities	Ozone

85 TOF: Tetralogy of Fallot; PM2.5: fine particulate matter

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87 **Table S6.** Differentially methylated regions (DMRs) mapped to genes that were differentially methylated by TOF status or air pollution exposure.

Gene	Gene Set		Gene	Gene Set		Gene	Gene Set
<i>ADAMTSL2</i>	TOF		<i>ADGB</i>	PM2.5		<i>BOLL</i>	Ozone
<i>AIRE</i>	TOF		<i>AKR1E2</i>	PM2.5		<i>CDK16</i>	Ozone
<i>ASB3</i>	TOF		<i>ANKRD30B</i>	PM2.5		<i>CENPQ</i>	Ozone
<i>CALCA</i>	TOF		<i>NR5A2</i>	PM2.5		<i>CFLAR</i>	Ozone
<i>CALCB</i>	TOF		<i>TBC1D22A</i>	PM2.5		<i>FSIP1</i>	Ozone
<i>CMTM2</i>	TOF		<i>TMEM176A</i>	PM2.5		<i>GFM2</i>	Ozone
<i>DLEU7</i>	TOF		<i>TNR</i>	PM2.5		<i>HLA-DPA1</i>	Ozone
<i>GEMIN6</i>	TOF		<i>TRAM1L1</i>	PM2.5		<i>HLA-DPB1</i>	Ozone
<i>GPR75</i>	TOF					<i>INSM2</i>	Ozone
<i>GSTM1</i>	TOF					<i>LYPD8</i>	Ozone
<i>GSTM2</i>	TOF					<i>MICB</i>	Ozone
<i>GSTM5</i>	TOF					<i>MUT</i>	Ozone
<i>NAV1</i>	TOF					<i>NR5A2</i>	Ozone
<i>PRPH</i>	TOF					<i>NSA2</i>	Ozone
<i>SKAP2</i>	TOF					<i>PACRG</i>	Ozone
<i>SMAD9</i>	TOF					<i>PPP3CA</i>	Ozone
<i>TTLL8</i>	TOF					<i>SLC6A15</i>	Ozone
<i>VENTX</i>	TOF					<i>SPATC1L</i>	Ozone
<i>ZNF692</i>	TOF					<i>SRSF8</i>	Ozone
						<i>STAC</i>	Ozone
						<i>TBC1D22A</i>	Ozone
						<i>THADA</i>	Ozone
						<i>TMEM176A</i>	Ozone
						<i>TRPC3</i>	Ozone
						<i>UBE2L3</i>	Ozone

88 TOF: Tetralogy of Fallot; PM2.5: Fine Particulate Matter

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