Supplemental Material

Supplemental Figure 1. Work flow. The included research subjects were from the University of Kentucky Alzheimer's Disease Center (UK-ADC) autopsy cohort. From each individual, cerebellum samples snap-frozen at autopsy were used to isolate DNA (for evaluation of HS-Aging risk alleles) and to produce brain extracts for thyroid hormone assays. Histopathologic evaluation included conventional neuropathologic assessments in accordance with the National Institutes on Aging-Alzheimer's Association consensus guideline recommendations for neuropathologic diagnosis of AD neuropathologic changes (Montine et al., 2012). Additional, digital neuropathologic methods were applied to assess the TDP-43 pathology in the same cases. Finally, the clinical, genetic, pathologic, and biochemical parameters were analyzed in association with each other to detect evidence of disease-driving mechanisms in brains harboring TDP-43 pathology.

Supplemental Figure 2. Representative primary data from SNP characterization using TaqMan® Predesigned SNP Genotyping Assays. Shown here are results for rs5848 (*GRN* risk allele). Note the clear differentiation between homozygote (GG and TT) and heterozygous (GT) cases. "NTC" refers to no template controls.

Supplemental Figure 3. Representative results of T4 and T3 ELISA assays. Brain extracts were analyzed on multiple 96-well ELISA microplates, according to manufacturer instructions. In order to account for variability between plates, an independent standard curve was performed on each plate and used to analyze the samples on that plate only. Standard data was fit to a 4-parameter logistic equation. Shown are independent standard replicates run for T4 (A) and T3 (B) for each set of samples, with manufacturer-provided standards. A representative curve fit is shown for the first set of samples analyzed. In the present study, the average detected level of T4 was approximately 4.8 μg/dL, and the average detected level of T3 was approximately 1.9 ng/mL, denoted on each graph with a red arrow. (Note that these values were standardized to tissue weight for reporting). These values of tissue T3 and T4 were within numerical ranges where the standard curve of this assay was relatively stable.

Supplemental Figure 4. Results of triiodothyronine (T3) and thyroxine (T4) assays from human brain parenchymal extracts, showing ELISA results from both superior middle and temporal (SMTG; Brodmann Areas 21 and 22) and cerebellum (CBL) across a range of Alzheimer's disease pathologic severity. These analyses were performed on a convenience subsample of cases (n=21) for which snap-frozen low-PMI biosamples were available from both SMTG and CBL regions. Note that there is a trend for more advanced Alzheimer's disease pathology (higher Braak NFT stages) to have lower T3 levels in SMTG, but not CBL. The implications of these results are currently unknown – it is possible that pathology affects the thyroid hormone levels (perhaps in association with disruption of blood-brain barrier), or, perhaps, the thyroid hormone levels influences the pathology.

Supplemental Figure 5. Results of triiodothyronine (T3) thyroxine (T4) assays from human brain parenchymal extracts, comparing T3 and T4 results from cerebellum. From each case (n=136 included), an extract was made from the cerebellum snap-frozen at autopsy, and then evaluated using enzyme-linked immunosorbent assay (ELISA)-based assay kits. Each data point represents an individual case. The T3 and T4 assays were run separately. There was a moderately strong correlation between the T3 and T4 readings for individual cases. ($r=0.50$, $p<0.0001$).

Supplemental Figure 6. Results of T3 and T4 ELISA assays on brain extracts (for analyses in the current study), stratified by factors that could indicate systematic technical bias. The two factors shown here are the number of years store at -80°C in freezers between autopsy and experimental assay (A,B) and the number of hours between death and post-mortem examination (C,D). Results for triiodothyronine (T3; A, C) and thyroxine (T4; B, D) are shown. Note that on all of these charts, the R^2 correlation coefficient is <0.02 and the regression probability statistic was $p>0.1$ for all of these, indicating a lack of impact detected for these particular technical factors.

Supplemental Figure 7. Summary figure. The analytic results are compatible with the hypothesis that TDP-43 pathology in aging is affected by interactions between a complex set of factors that include genetic risk alleles and comorbid pathologies, particularly advanced Alzheimer's disease. Distinct risk alleles appear to affect different disease-affecting nodes and pathways. The current study is compatible with the hypothesis that transport of thyroid hormone from the blood (thyroxine, or T4), and conversion into active trioiodothyronine (T3) is a possible compensatory mechanism that is affected by gene variants near the main brain thyroid hormone transporter gene.

Supplemental Tables

Supplemental Table 1. Clinical, genetic, pathologic, and biochemical features of the present cohort (n=136), stratified by *TMEM106B* risk allele (SNP rs1990622) status

	rs1990622 status (*TMEM106B* gene)			p-value: risk vs non-risk allele(s)
	AA	**AG**	**GG**	
n	55	58	23	NA
Age at death, Avg	84.1	83.6	84.7	0.960
% Female	56.4	51.7	73.9	0.078
% Normal cognition/clinical state	26.4	31.0	30.4	0.877
% Demented cognition/clinical state	54.7	63.8	43.5	0.159
MMSE, Avg	19.8	19.7	23.3	0.067
% *SLCO1A2/IAPP* risk allele(s)	29.1	15.5	34.8	0.197
% *GRN* risk allele(s)	38.2	50.0	26.1	0.107
% *TMEM106B* risk allele(s)	100.0	100.0	0.0	NA
% *ABCC9* risk allele(s)	23.6	22.4	21.7	0.895
% *APOE* ε4 allele [+]	30.0	33.3	27.3	0.689
% Braak NFT stage V or VI	43.6	51.7	60.9	0.253
% HS-Aging	27.3	29.3	21.7	0.518
DG: % [+] TDP-43 inclusions	33	33	13	0.147
DG: median # inclusions in [+] cases	4.5	2	5	
CA1: % [+] TDP-43 inclusions	40	40	22	0.234
CA1: median # inclusions in [+] cases	6.5	7	1	
CA1 TDP-43 neurites, median	1.96E-03	1.01E-03	2.49E-03	0.605
Sub: % [+] TDP-43 inclusions	33	33	13	0.534
Sub: median # inclusions in [+] cases	9.5	14	6	
Sub TDP-43 neurites, median	2.42E-03	1.67E-03	3.20E-03	0.922
% with Frontal Cx TDP-43	7.3	5.2	0	0.602
T4 fg/mg brain tissue, Avg	189.17	199.5	188.06	0.682
T3 fg/mg brain tissue, Avg	7.6	7.59	8	0.539
Average of T3/T4 ratio, Avg	0.040	0.038	0.043	0.145
PMI (hrs), Avg	4.6	5.2	3.4	0.505

*- *APOE* genotype was available on 120/136 (88.2%) of the included subjects, and final MMSE scores were available for 126/136 (92.6%) of subjects. N/A: Not applicable.

Supplemental Table 2. Clinical, genetic, pathologic, and biochemical features of the present cohort (n=136), stratified by *GRN* risk allele (SNP rs5848) status

	rs5848 status (*GRN* gene)			p-value: risk vs non-risk allele(s)
	CC	**CT**	**TT**	
n	80	41	15	N/A
Age at death, Avg	84.53	83.29	82.73	0.211
% Female	61.2	53.7	46.7	0.272
% Normal cognition/clinical state	30.4	22.5	40	0.697
% Demented cognition/clinical state	54.4	65	46.7	0.522
MMSE, Avg*	21.1	19.26	19.5	0.319
% *SLCO1A2/IAPP* risk allele(s)	27.5	17.1	26.7	0.293
% *GRN* risk allele(s)	0	100	100	N/A
% *TMEM106B* risk allele(s)	78.8	87.8	93.3	0.107
% *ABCC9* risk allele(s)	25	17.1	26.7	0.464
% *APOE* ε4 allele [+]*	21.4	48.6	33.3	**0.008**
% Braak NFT stage V or VI*	46.2	56.1	53.3	0.296
% HS-Aging	25	29.3	33.3	0.490
DG: % [+] TDP-43 inclusions	25	32	47	0.323
DG: median # inclusions in [+] cases	5.5	2	1	
CA1: % [+] TDP-43 inclusions	31	34	73	0.209
CA1: median # inclusions in [+] cases	7	7.5	6	
CA1 TDP-43 neurites, median	1.44E-3	1.40E-3	3.13E-3	0.689
Sub: % [+] TDP-43 inclusions	25	32	47	0.978
Sub: median # inclusions in [+] cases	13	15	7.5	
Sub TDP-43 neurites, median	2.15E-3	1.90E-3	2.42E-3	0.663
% with Frontal Cx TDP-43	6.2	4.9	0	0.700
T4 fg/mg brain tissue, Avg	197.6	188.92	183.11	0.294
T3 fg/mg brain tissue, Avg	7.71	7.29	8.47	0.852
Average of T3/T4 ratio, Avg	0.039	0.039	0.046	0.370
PMI (hrs), Avg	4.8	3.7	6.68	0.817

*- *APOE* genotype was available on 120/136 (88.2%) of the included subjects, and final MMSE scores were available for 126/136 (92.6%) of subjects. N/A: Not applicable.

Supplemental Table 3. Clinical, genetic, pathologic, and biochemical features of the present cohort (n=136), stratified by *ABCC9* risk allele (SNP rs704180) status

	rs704180 status (*ABCC9* gene)			p-value: risk vs non-risk allele(s)
	AA	**AG**	**GG**	
n	31	75	30	N/A
Age at death, Avg	82.55	84.92	83	0.724
% Female	64.5	58.7	46.7	0.359
% Normal cognition/clinical state	35.5	28.4	24.1	0.372
% Demented cognition/clinical state	54.8	58.1	55.2	0.810
MMSE, Avg*	19.11	20.01	22.52	0.506
% *SLCO1A2/IAPP* risk allele(s)	22.6	25.3	23.3	0.803
% *GRN* risk allele(s)	35.5	42.7	43.3	0.464
% *TMEM106B* risk allele(s)	83.9	78.7	93.3	0.895
% *ABCC9* risk allele(s)	100	0	0	N/A
% *APOE* ε4 allele [+]*	29.2	28.6	38.5	0.843
% Braak NFT stage V or VI*	41.9	53.3	50	0.307
% HS-Aging	32.3	28.0	20.0	0.472
DG: % [+] TDP-43 inclusions	32	32	20	0.847
DG: median # inclusions in [+] cases	2	5	4	
CA1: % [+] TDP-43 inclusions	45	37	27	0.153
CA1: median # inclusions in [+] cases	2.5	8.5	11	
CA1 TDP-43 neurites, median	1.15E-3	2.47E-3	1.25E-3	0.140
Sub: % [+] TDP-43 inclusions	32	32	20	0.903
Sub: median # inclusions in [+] cases	5	9	17	
Sub TDP-43 neurites, median	1.93E-3	1.99E-3	2.93E-3	0.460
% with Frontal Cx TDP-43	9.7	5.3	0	0.195
T4 fg/mg brain tissue, Avg	184.21	199.77	186.92	0.231
T3 fg/mg brain tissue, Avg	6.9	7.97	7.69	0.074
Average of T3/T4 ratio, Avg	0.037	0.040	0.041	0.156
PMI (hrs), Avg	6.3	4.0	4.3	0.060

*- *APOE* genotype was available on 120/136 (88.2%) of the included subjects, and final MMSE scores were available for 126/136 (92.6%) of subjects. N/A: Not applicable.

Supplemental Table 4. Clinical, genetic, pathologic, and biochemical features of the present cohort (n=136), stratified by *SLCO1A2/IAPP* risk allele (SNP rs12301085, a proxy for rs73069071, identified by Roostaei et al (Roostaei et al., 2016)) status.

	rs12496790 status (*SLCO1A2/IAPP* genes)			p-value: risk vs non-risk allele(s)
	AA	**AG**	**GG**	
N	2	31	103	N/A
Age at death, Avg	72.0	85.8	83.6	0.142
% Female	50	58.1	57.3	0.976
% Normal cognition/clinical state	50	33.3	27.5	0.452
% Demented cognition/clinical state	50	46.7	59.8	0.198
MMSE, Avg*	17.5	22.2	19.82	0.258
% *SLCO1A2/IAPP* risk allele(s)	100	100	0	N/A
% *GRN* risk allele(s)	50	32.3	43.7	0.293
% *TMEM106B* risk allele(s)	100	100	0	0.197
% *ABCC9* risk allele(s)	50	19.4	23.3	0.895
% *APOE* ε4 allele [+]*	0	18.5	35.2	0.069
% Braak NFT stage V or VI*	0	38.7	54.4	0.072
% HS-Aging	0	29	27.2	0.992
DG: % [+] TDP-43 inclusions	0	29	30	0.829
DG: median # inclusions in [+] cases	0	6	2	
CA1: % [+] TDP-43 inclusions	0	35	38	0.894
CA1: median # inclusions in [+] cases	0	4	7	
CA1 TDP-43 neurites, median	8.41E-3	2.00E-3	1.44E-3	0.286
Sub: % [+] TDP-43 inclusions	0	29	30	0.66
Sub: median # inclusions in [+] cases	0	16	7	
Sub TDP-43 neurites, median	1.57E-3	1.97E-3	2.25E-3	0.934
% with Frontal Cx TDP-43	0	3.2	5.8	1
T4 fg/mg brain tissue, Avg	186.36	201.76	191	0.384
T3 fg/mg brain tissue, Avg	6.25	7.1	7.86	0.134
Average of T3/T4 ratio, Avg	0.034	0.035	0.041	0.058
PMI (hrs), Avg	2.9	5.0	4.7	0.362

*- *APOE* genotype was available on 120/136 (88.2%) of the included subjects, and final MMSE scores were available for 126/136 (92.6%) of subjects. N/A: Not applicable.

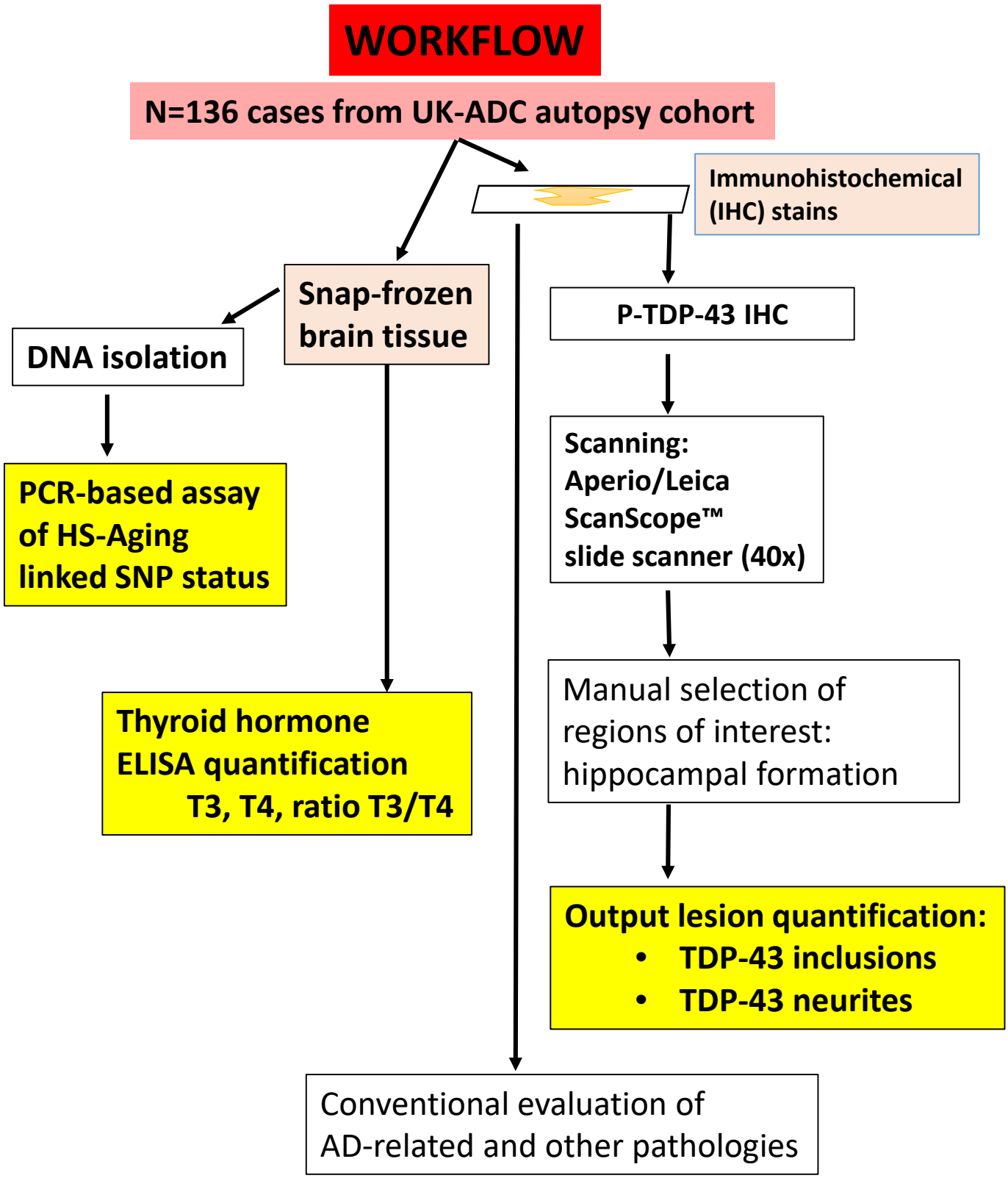
Supplemental Table 5. Clinical, genetic, pathologic, and biochemical features of the present cohort (n=136), stratified by *KCNMB2* risk allele (SNP rs12496790, a proxy for rs9637454) status. The risk allele for rs12496790 would be "AA or AG" according to Beecham et al (Beecham et al., 2014).

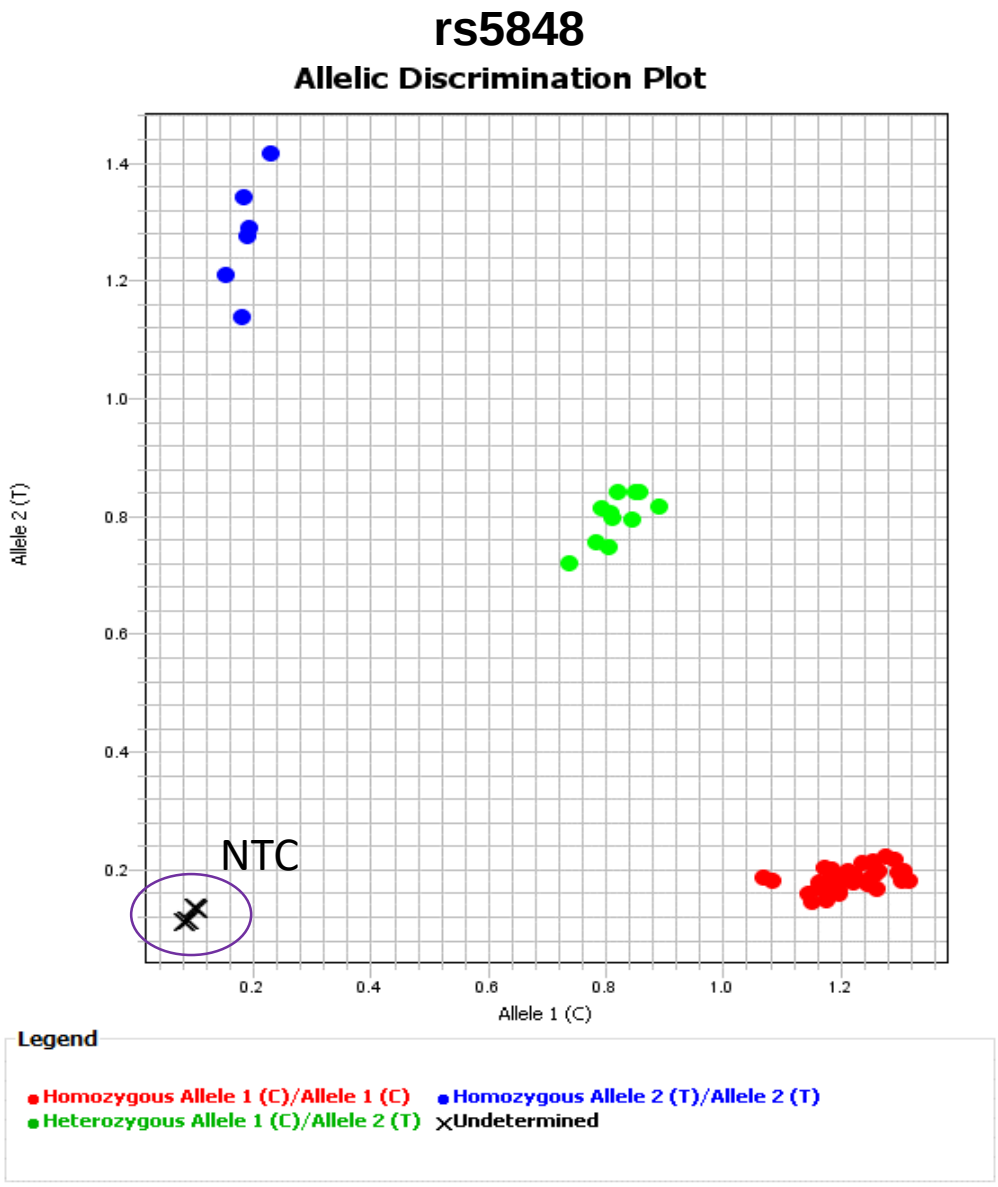
	rs12496790 status *(KCNMB2* gene)			p-value: risk vs non-risk allele(s)
	AA	**AG**	**GG**	
n	7	57	72	N/A
Age at death, Avg	85.3	83.5	84.2	0.693
% Female	57.1	57.9	56.9	1
% Normal cognition/clinical state	28.6	30.4	28.2	1
% Demented cognition/clinical state	42.9	55.4	59.2	0.466
MMSE, Avg*	21.57	20.35	20.22	0.742
% *SLCO1A2/IAPP* risk allele(s)	42.9	28.1	19.4	0.360
% *GRN* risk allele(s)	0	33.3	51.4	**0.041**
% *TMEM106B* risk allele(s)	100	0	0	0.337
% *ABCC9* risk allele(s)	14.3	31.6	16.7	1
% *APOE* ε4 allele [+]*	33.3	26	34.4	1
% Braak NFT stage V or VI*	28.6	54.4	48.6	0.441
% HS-Aging	14.3	31.6	25	0.674
DG: % [+] TDP-43 inclusions	14	30	31	0.673
DG: median # inclusions in [+] cases	1	3	3	
CA1: % [+] TDP-43 inclusions	14	44	33	0.407
CA1: median # inclusions in [+] cases	13	3	7.5	
CA1 TDP-43 neurites, median	8.38E-3	1.76E-3	1.42E-3	0.883
Sub: % [+] TDP-43 inclusions	14	30	31	0.558
Sub: median # inclusions in [+] cases	21	6	16.5	
Sub TDP-43 neurites, median	2.00E-3	1.99E-3	2.34E-3	0.832
% with Frontal Cx TDP-43	0	8.8	2.8	1
T4 fg/mg brain tissue, Avg	190.1	199.1	189.2	0.871
T3 fg/mg brain tissue, Avg	6.77	7.97	7.51	0.229
Average of T3/T4 ratio, Avg	0.036	0.040	0.040	0.566
PMI (hrs), Avg	4.5	5.1	4.5	0.987

*- *APOE* genotype was available on 120/136 (88.2%) of the included subjects, and final MMSE scores were available for 126/136 (92.6%) of subjects. N/A: Not applicable.

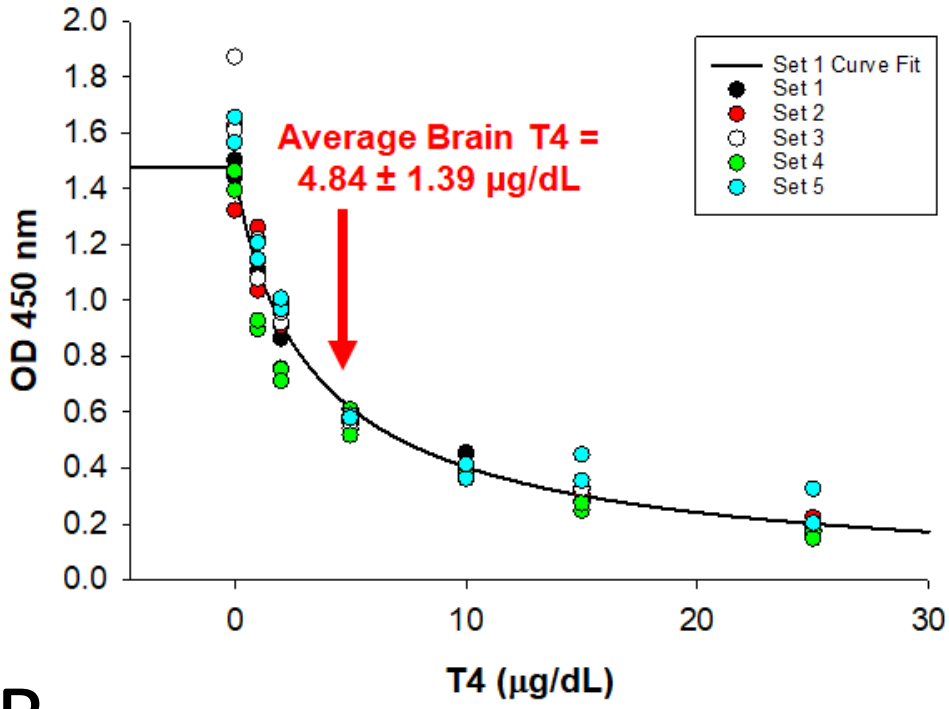
Supplemental Spreadsheet

Additional information about evaluated SNPs and PCR-based SNP assay kits is presented in Excel spreadsheet format


WORKFLOW
N=136 cases from UK-ADC autopsy cohort
Immunohistochemical (IHC) stains
Snap-frozen brain tissue
DNA isolation
PCR-based assay of HS-Aging linked SNP status
Thyroid hormone ELISA quantification T3, T4, ratio T3/T4
P-TDP-43 IHC
Scanning: Aperio/Leica ScanScope™ slide scanner (40x)
Manual selection of regions of interest: hippocampal formation
Output lesion quantification:
• TDP-43 inclusions
• TDP-43 neurites
Conventional evaluation of AD-related and other pathologies


rs5848
Allelic Discrimination Plot
Allele 2 (T)
Allele 1 (C)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.2
0.4
0.6
0.8
1.0
1.2
NTC
Legend
Homozygous Allele 1 (C)/Allele 1 (C)
Homozygous Allele 2 (T)/Allele 2 (T)
Heterozygous Allele 1 (C)/Allele 2 (T)
Undetermined

A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Set 1
Set 2
Set 3
Set 4
Set 5
OD 450 nm
T4 (µg/dL)
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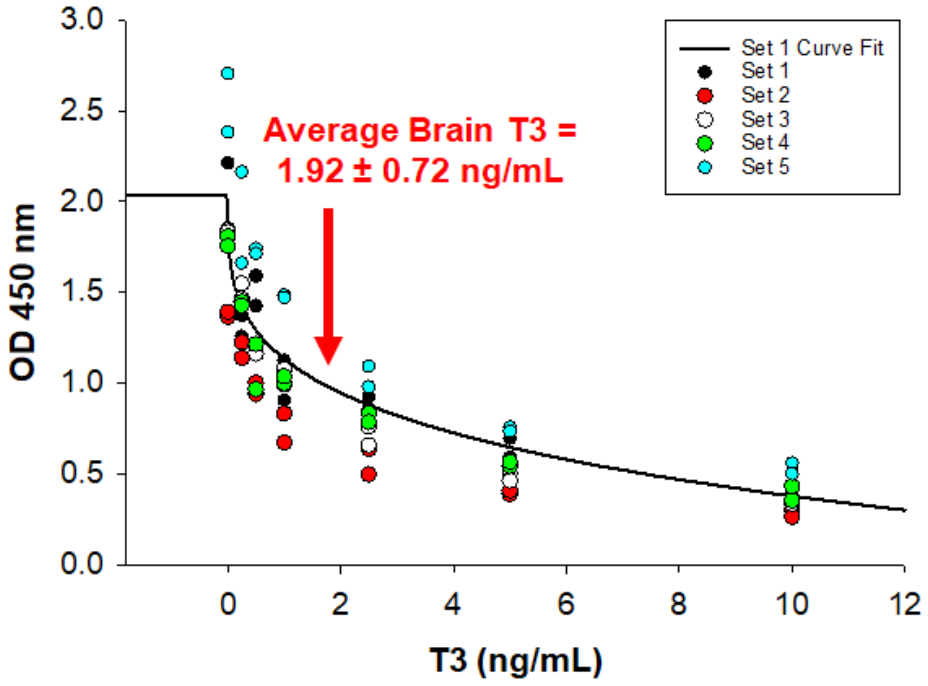
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
0
10
20
30

B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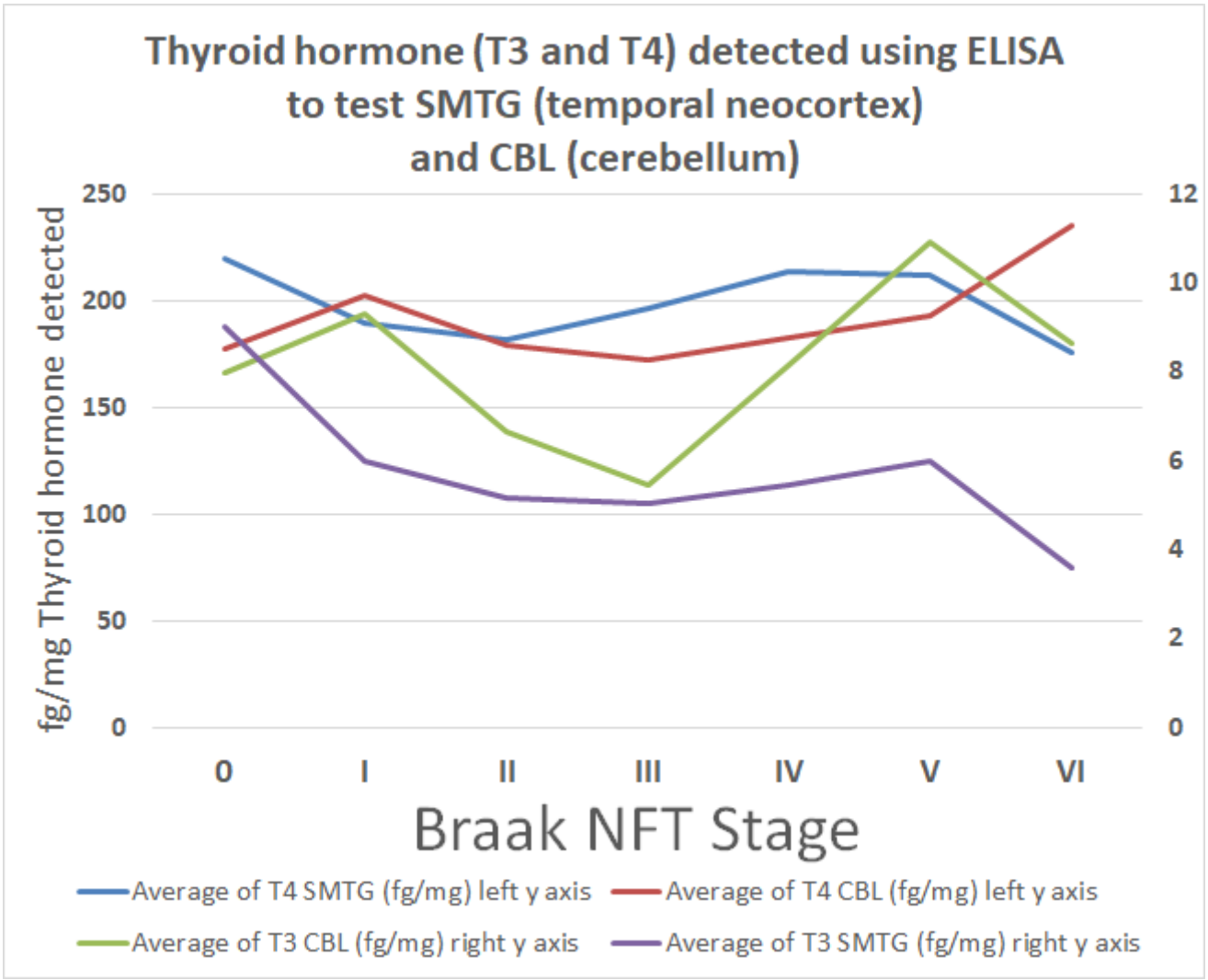
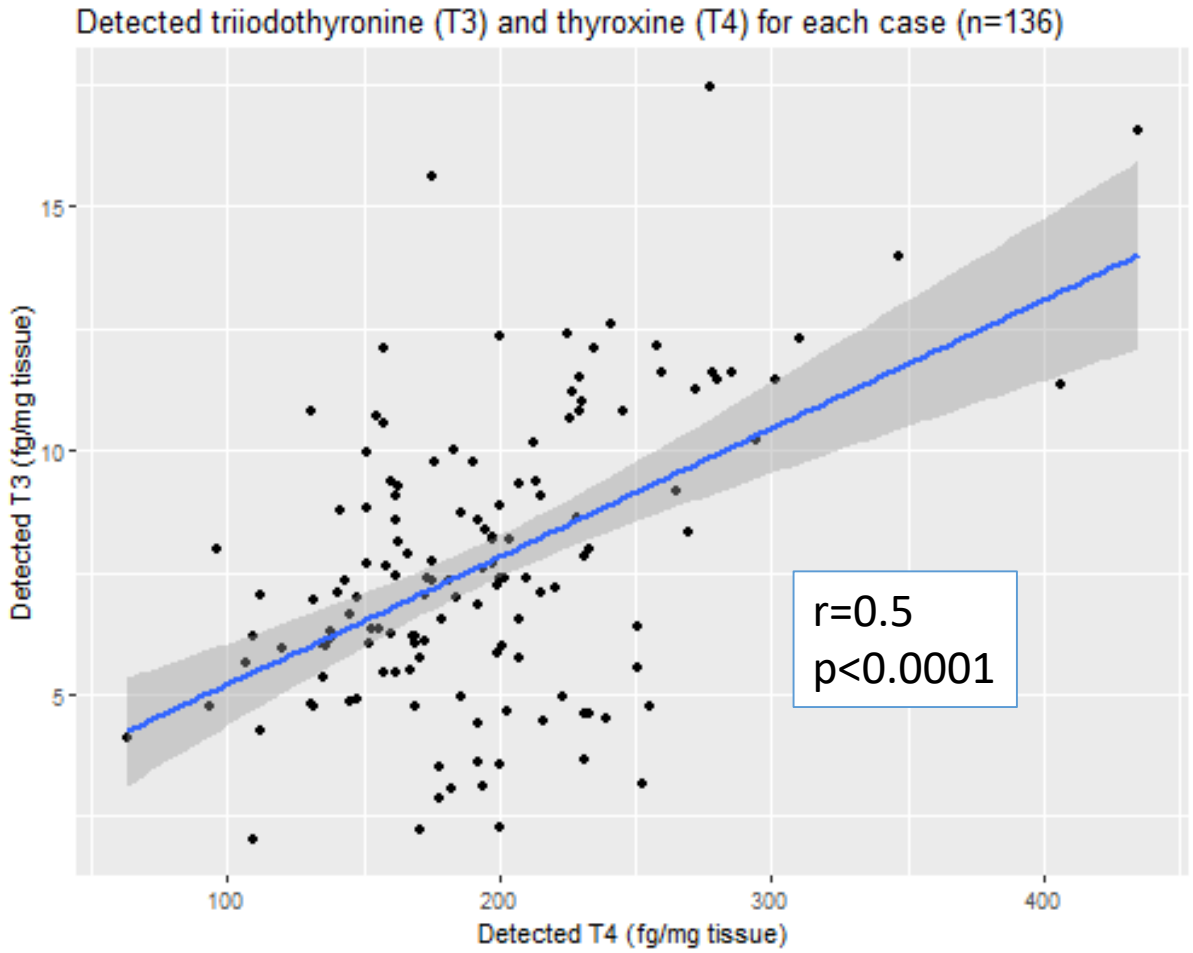
1.92 ± 0.72 ng/mL
Set 1 Curve Fit
Set 1
Set 2
Set 3
Set 4
Set 5
OD 450 nm
T3 (ng/mL)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
2
4
6
8
10
12

Nelson PT et al, Supplemental Fig. 4



Nelson PT et al, Supplemental Fig. 5



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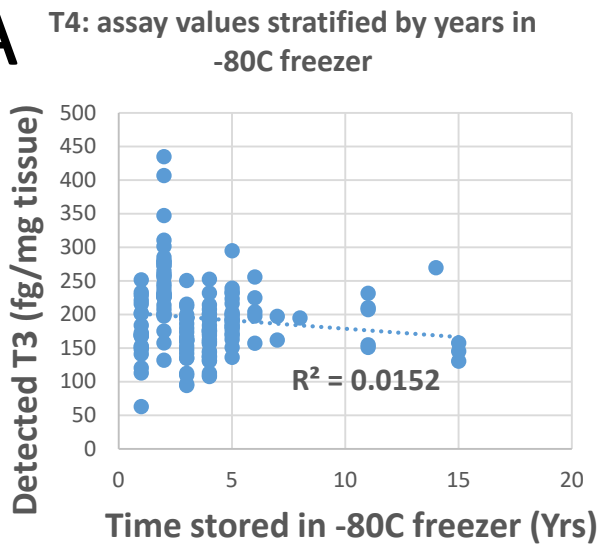
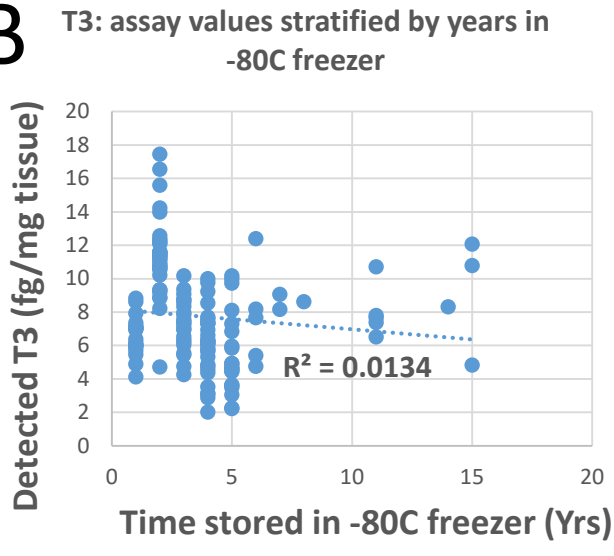
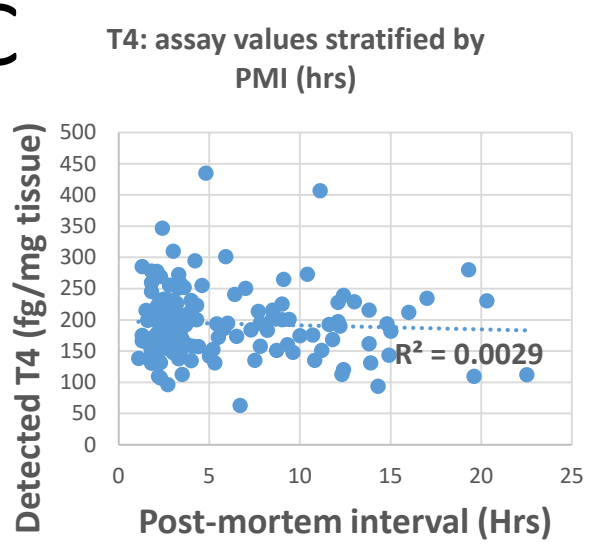

T4: assay values stratified by years in -80C freezer
Detected T3 (fg/mg tissue)
500
450
400
350
300
250
200
150
100
50
0
0
5
10
15
20
R² = 0.0152
Time stored in -80C freezer (Yrs)

B


T3: assay values stratified by years in -80C freezer
Detected T3 (fg/mg tissue)
20
18
16
14
12
10
8
6
4
2
0
0
5
10
15
20
R² = 0.0134
Time stored in -80C freezer (Yrs)

C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Detected T4 (fg/mg tissue)
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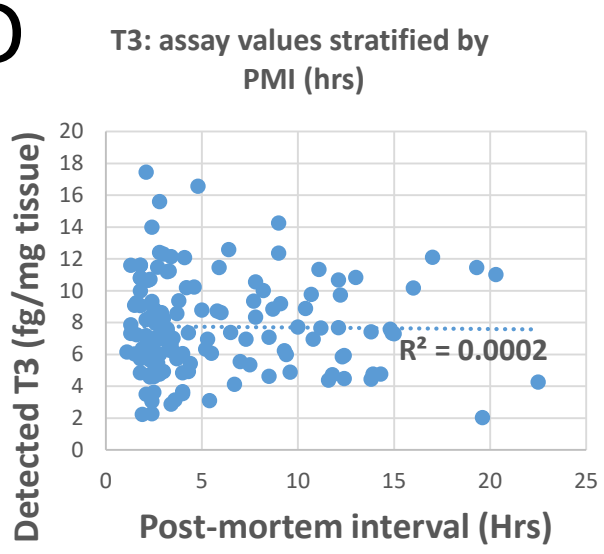
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
R² = 0.0029
Post-mortem interval (Hrs)

D


T3: assay values stratified by PMI (hrs)
Detected T3 (fg/mg tissue)
20
18
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
R² = 0.0002
Post-mortem interval (Hrs)

Nelson PT et al, Supplemental Fig. 7