**Submission to Respiratory Research – ONLINE DATA SUPPLEMENT**

May 13th 2018

***SERPINA1* Methylation and Lung Function in Tobacco-Smoke Exposed European Children and Adults: a Meta-Analysis of ALEC population-based cohorts.**

Anna Beckmeyer-Borowko\*, Medea Imboden\*, Faisal I. Rezwan\*, Matthias Wielscher\*, Andre F.S. Amaral\*, Ayoung Jeong, Emmanuel Schaffner, Juha Auvinen, Sylvain Sebert, Ville Karhunen, Robert Bettschart, Alexander Turk, Marco Pons, Daiana Stolz, Florian Kronenberg, Ryan Arathimos, Gemma C Sharp, Caroline Relton, Alexander J. Henderson, Marjo-Riitta Jarvelin\*\*, Deborah Jarvis\*\*, John W. Holloway\*\*, Nicole M. Probst-Hensch\*\*

\*equal first author contribution; \*\*equal senior author contribution



Table ES1: Meta-Analysis of the association of methylation at CpGs in the *SERPINA\** Gene Cluster with FVC level and decline in adult ever smokers from SAPALDIA, ECRHS and NFBC (n=1076) and with circulating AAT concentrations in SAPALDIA (n=561)

\*From *SERPINA* gene cluster located on chromosome 14 between positions: 94’641’781 and 95’235’125 (human genome build 37/hg19).
† Nominal P-values, Bonferroni corrected significance level for 119 tests is P-value 4.6x10-4
‡Direction of effect for SAPALDIA, ECRHS and NFBC. A positive sign indicates that an increase in methylation is associated with higher level of lung function (cross-sectional models) and with an attenuation of lung function decline, respectively (change model).
Cross-Sectional and repeat cross-sectional models were adjusted for: study center, age, age2, education, height, height2, sex, sex\*age, (sex\*age)2, sex\*height, (sex\*height)2, Bcell, CD4T, CD8T, Eos, Mono, NK. Repeat cross-sectional in addition ran with a random intercept on the subject. In predictive models, annual change in lung function was computed as the difference between T2 and T1 divided by time of follow-up: (T2-T1)/follow-up models adjusted for covariates from T1
Sample size for circulating AAT for SAPALDIA n= 561, cross-sectional and prediction models (change) n=1076, and n=1122 for repeat cross-sectional models
Significant CpGs at the nominal level are bolded

Table ES2: Meta-Analysis of the association of methylation at 119 CpGs in the *SERPINA*\* cluster with FEV1 level and decline in adult ever smokers from SAPALDIA, ECRHS and NFBC (n=1076) and with circulating AAT concentrations in SAPALDIA (n=561)





\*From *SERPINA* gene cluster located on chromosome 14 between positions: 94’641’781 and 95’235’125 (human genome build 37/hg19).
† Nominal P-values, Bonferroni corrected significance level for 119 tests is P-value 4.6x10-4
‡Direction of effect for SAPALDIA, ECRHS and NFBC. A positive sign indicates that an increase in methylation is associated with higher level of lung function (cross-sectional models) and with an attenuation of lung function decline, respectively (change model).
Cross-Sectional and repeat cross-sectional models were adjusted for: study center, age, age2, education, height, height2, sex, sex\*age, (sex\*age)2, sex\*height, (sex\*height)2, Bcell, CD4T, CD8T, Eos, Mono, NK. Repeat cross-sectional in addition ran with a random intercept on the subject. In predictive models, annual change in lung function was computed as the difference between T2 and T1 divided by time of follow-up: (T2-T1)/follow-up models adjusted for covariates from T1
Sample size for circulating AAT for SAPALDIA n= 561, cross-sectional and prediction models (change) n= 1076, and n= 1122 for repeat cross-sectional models
*SERPINA1* CpGs are highlighted in bold, italic. Nominally significant CpGs are in addition highlighted in grey
Significant CpGs at the nominal level, outside *SERPINA1* gene, are bolded

Table ES3: Meta-analysis of the association of methylation at 119 CpGs in the *SERPINA*\* gene cluster with FVC level and decline in adult ever smokers from SAPALDIA, ECRHS and NFBC (n=1076) and with circulating AAT concentrations in SAPALDIA (n=561)





\*From *SERPINA* gene cluster located on chromosome 14 between positions: 94’641’781 and 95’235’125 (human genome build 37/hg19).
† Nominal P-values, Bonferroni corrected significance level for 119 tests is P-value 4.6x10-4
‡Direction of effect for SAPALDIA, ECRHS and NFBC. A positive sign indicates that an increase in methylation is associated with higher level of lung function (cross-sectional models) and with an attenuation of lung function decline, respectively (change model).
Cross-Sectional and repeat cross-sectional models were adjusted for: study center, age, age2, education, height, height2, sex, sex\*age, (sex\*age)2, sex\*height, (sex\*height)2, Bcell, CD4T, CD8T, Eos, Mono, NK. Repeat cross-sectional in addition ran with a random intercept on the subject. In predictive models, annual change in lung function was computed as the difference between T2 and T1 divided by time of follow-up: (T2-T1)/follow-up models adjusted for covariates from T1
Sample size for circulating AAT for SAPALDIA n= 561, cross-sectional and prediction models (change) n= 1076, and n= 1122 for repeat cross-sectional models
*SERPINA1* CpGs are highlighted in bold, italic. Nominally significant CpG are in addition highlighted in grey
Significant CpGs at the nominal level, outside *SERPINA1* gene, are bolded

Table ES4: Meta-analysis of the association of methylation at 119 CpGs in the *SERPINA*\* gene cluster with FEV1/FVC level and decline in adult ever smokers from SAPALDIA, ECRHS and NFBC (n=1076) and with circulating AAT concentrations in SAPALDIA (n=561)





\*From *SERPINA* gene cluster located on chromosome 14 between positions: 94’641’781 and 95’235’125 (human genome build 37/hg19).
† Nominal P-values, Bonferroni corrected significance level for 119 tests is P-value 4.6x10-4
‡Direction of effect for SAPALDIA, ECRHS and NFBC. A positive sign indicates that an increase in methylation is associated with higher level of lung function (cross-sectional models) and with an attenuation of lung function decline, respectively (change model).
Cross-Sectional and repeat cross-sectional models were adjusted for: study center, age, age2, education, height, height2, sex, sex\*age, (sex\*age)2, sex\*height, (sex\*height)2, Bcell, CD4T, CD8T, Eos, Mono, NK. Repeat cross-sectional in addition ran with a random intercept on the subject. In predictive models, annual change in lung function was computed as the difference between T2 and T1 divided by time of follow-up: (T2-T1)/follow-up models adjusted for covariates from T1
Sample size for circulating AAT for SAPALDIA n= 561, cross-sectional and prediction models (change) n= 1076, and n= 1122 for repeat cross-sectional models
*SERPINA1* CpGs are highlighted in bold, italic. Nominally significant CpGs are in addition highlighted in grey
Significant CpGs at the nominal level, outside *SERPINA1* gene, are bolded.
cg08257009 is bolded and highlighted in dark grey. This is the only CpG in the *SERPINA* cluster that withstood Bonferroni-correction for multiple testing.



Table ES5: Association of methylation at 119 CpGs in the *SERPINA*\* gene cluster with FEV1 level and decline in ALSPAC children exposed to tobacco smoke (n=259)



\*From *SERPINA* gene cluster located on chromosome 14 between positions: 94’641’781 and 95’235’125 (human genome build 37/hg19).
† Children and adolescents exposed to tobacco-smoke defined as: mother smoked during pregnancy and/or lived with a smoker and/or reported smoking ≥twice in their lifetime
‡ Nominal P-values, Bonferroni corrected significance level for 119 tests is P-value 4.6x10-4
Cross-Sectional and repeat cross-sectional models were adjusted for: study center, age, mother education, height, (height-mean(height))2, sex, sex\*age, sex\*height, and cell composition (CD8T; CD4T; NK; Bcell; Mono; Eos). Repeat cross-sectional in addition ran with a random intercept on the subject. In predictive models, annual change in lung function was computed as the difference between T2 and T1 divided by time of follow-up: (T2-T1)/follow-up models adjusted for covariates from T1
*SERPINA1* CpGs are highlighted in bold, italic. Nominally significant CpGs are in addition highlighted in grey
Significant CpGs at the nominal level, outside *SERPINA1* gene, are bolded.



Table ES6: Association of methylation at 119 CpGs in the *SERPINA*\* gene cluster with FVC level and decline in in ALSPAC children exposed to tobacco smoke (n=259)



\*From *SERPINA* gene cluster located on chromosome 14 between positions: 94’641’781 and 95’235’125 (human genome build 37/hg19).
† Children and adolescents exposed to tobacco-smoke defined as: mother smoked during pregnancy and/or lived with a smoker and/or reported smoking ≥twice in their lifetime
‡ Nominal P-values, Bonferroni corrected significance level for 119 tests is P-value 4.6x10-4
Cross-Sectional and repeat cross-sectional models were adjusted for: study center, age, mother education, height, (height-mean(height))2, sex, sex\*age, sex\*height, and cell composition (CD8T; CD4T; NK; Bcell; Mono; Eos). Repeat cross-sectional in addition ran with a random intercept on the subject. In predictive models, annual change in lung function was computed as the difference between T2 and T1 divided by time of follow-up: (T2-T1)/follow-up models adjusted for covariates from T1
*SERPINA1* CpGs are highlighted in bold, italic. Nominally significant CpGs are in addition highlighted in grey
Significant CpGs at the nominal level, outside *SERPINA1* gene, are bolded.



Table ES7: Association of methylation at 119 CpGs in the *SERPINA*\* gene cluster with FEV1/FVC level and decline in ALSPAC children exposed to tobacco smoke (n=259)



\*From *SERPINA* gene cluster located on chromosome 14 between positions: 94’641’781 and 95’235’125 (human genome build 37/hg19).
† Children and adolescents exposed to tobacco-smoke defined as: mother smoked during pregnancy and/or lived with a smoker and/or reported smoking ≥twice in their lifetime
‡ Nominal P-values, Bonferroni corrected significance level for 119 tests is P-value 4.6x10-4
Cross-Sectional and repeat cross-sectional models were adjusted for: study center, age, mother education, height, (height-mean(height))2, sex, sex\*age, sex\*height, and cell composition (CD8T; CD4T; NK; Bcell; Mono; Eos). Repeat cross-sectional in addition ran with a random intercept on the subject. In predictive models, annual change in lung function was computed as the difference between T2 and T1 divided by time of follow-up: (T2-T1)/follow-up models adjusted for covariates from T1
*SERPINA1* CpGs are highlighted in bold, italic. Nominally significant CpGs are in addition highlighted in grey
Significant CpGs at the nominal level, outside *SERPINA1* gene, are bolded.

Table ES8: Association of methylation at 119 CpGs in the *SERPINA*\* gene cluster with FEV1 level and decline in adult smokers from SAPALDIA, basic adjustment and adjustment for PIS and PIZ genotypes





\*From *SERPINA* gene cluster located on chromosome 14 between positions: 94’641’781 and 95’235’125 (human genome build 37/hg19).
† Nominal P-values, Bonferroni corrected significance level for 119 tests is P-value 4.6x10-4
‡Direction of effect for SAPALDIA, ECRHS and NFBC. A positive sign indicates that an increase in methylation is associated with higher level of lung function
 (cross-sectional models) and with an attenuation of lung function decline, respectively (change model).
Repeat cross-sectional models were adjusted for: study center, age, age2, education, height, height2, sex, sex\*age, (sex\*age)2, sex\*height, (sex\*height)2, Bcell, CD4T, CD8T, Eos, Mono, NK, random intercept on the subject. In predictive models, annual change in lung function was computed as the difference between T2 and T1 divided by time of follow-up: (T2-T1)/follow-up models adjusted for covariates from T1
SERPINA1 CpGs are highlighted in bold, italic. Nominally significant CpG are in addition highlighted in grey
Significant CpGs at the nominal level, outside *SERPINA1* gene, are bolded

Table ES9: Association of methylation at 119 CpGs in the *SERPINA*\* gene cluster with FEV1/FVC level and decline in adult smokers from SAPALDIA, basic adjustment and adjustment for PIS and PIZ genotypes





\*From *SERPINA* gene cluster located on chromosome 14 between positions: 94’641’781 and 95’235’125 (human genome build 37/hg19).
† Nominal P-values, Bonferroni corrected significance level for 119 tests is P-value 4.6x10-4
‡Direction of effect for SAPALDIA, ECRHS and NFBC. A positive sign indicates that an increase in methylation is associated with higher level of lung function
 (cross-sectional models) and with an attenuation of lung function decline, respectively (change model)
Repeat cross-sectional models were adjusted for: study center, age, age2, education, height, height2, sex, sex\*age, (sex\*age)2, sex\*height, (sex\*height)2, Bcell, CD4T, CD8T, Eos, Mono, NK, random intercept on the subject. In predictive models, annual change in lung function was computed as the difference between T2 and T1 divided by time of follow-up: (T2-T1)/follow-up models adjusted for covariates from T1
SERPINA1 CpGs are highlighted in bold, italic. Nominally significant CpG are in addition highlighted in grey
Significant CpGs at the nominal level, outside *SERPINA1* gene, are bolded