

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Håland G, Lødrup Carlsen KC, Sandvik L, et al. Reduced lung function at birth and the risk of asthma at 10 years of age. *N Engl J Med* 2006;355:1682-9.

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## SUPPLEMENTARY APPENDIX

### SUBJECTS AND METHODS

#### *General Study Design*

Initially, 3754 children were included into the birth cohort, representing 75 percent of all eligible subjects.(1) Inclusion criteria included: birth weight  $\geq 2000$  grams, gestational age  $\geq 37$  weeks, no serious illness that could impair respiration (severe respiratory, cardiovascular, neuromuscular, or metabolic diseases), no assisted ventilation or oxygen therapy after six hours of life, township of Oslo, and sufficient parental Norwegian language skills to answer extensive questionnaires every six months during the first two years of life.

All children were assessed every six months from birth to two years of age with questionnaires including details of environmental exposures, health of the index child, use of medication and of health care, as well as the health of household members.

A nested case-control study established included two clinical investigations between 0 and 2 years to ensure detailed examinations of children with recurrent ( $\geq 2$ ) or persistent ( $>4$  weeks) doctor confirmed bronchial obstruction (rBO) (n=306) and age-matched controls (n=306) (the child without lower respiratory illness born closest in time to the case).(2) At least one investigation was performed in 562 (91.8 percent) children. The attendance rate at the two-year visit was 84 percent (n=516), equally distributed between cases and controls.

Of the 1215 children with lung function measured at birth (n=803) and/or a clinical investigation by two years of age, 1019 (84 percent) attended the 10-year follow-up study(3) between September 2001 and July 2004. Investigations included detailed parental structured interview, blood tests, skin prick tests for allergic sensitisation, forced flow-volume loops, metacholine challenge test, exhaled nitric oxide, urine sampling and clinical examinations on day one, and an exercise test by treadmill running within one week. Dust samples were

collected by parents from the child's mattresses by vacuum-cleaner filters in a standardized manner. The present study includes the 614 children with lung function measured at birth attending the 10-year follow-up study.

#### *Methods at Age 10 Years*

Lung function was measured by forced expiratory flow volume loops according to European standard,(4) with the reference values of Zapletal,(5) using a SensorMedics Vmax 20c (SensorMedics Diagnostics, Yorba Linda, CA, USA). The reported values of forced expiratory flow in one second (FEV1) and forced mid-expiratory flow (FEF50) were the best baseline test obtained before challenge testing (bronchial challenge test with metacholine or treadmill test, both on separate days).(3)

Bronchial hyper responsiveness (BHR) measurements by metacholine provocation was performed according to international guidelines,(6) with maximum cumulated dose of 22.4  $\mu$ mol metacholine.

A standardized exercise test(7) was performed on the second day (after withholding short and long acting  $\beta$ -2 agonists for at least 12 and 48 hours, respectively, and leukotrine antagonists for 72 hours). Children ran on a treadmill at 5.5 percent incline for 6-8 minutes, the last four minutes running with 95 percent of estimated maximal heart rate. After a 20 minute observation time following the running, nebulized salbutamol was given in a dose of 0.1 ml per 10 kg bodyweight of a 5 mg/ml solution. FEV1 was measured 15 minutes thereafter. The exercise test was considered positive with a fall in FEV1  $\geq$ 10 percent of baseline FEV1 3-20 minutes after running ceased.

Skin prick tests (SPT) to common inhalant and food allergens were performed in 608/614 children with Soluprick® allergens (ALK Albello, Hørsholm, Denmark). Sensitization was considered positive with a wheal diameter  $\geq$  3mm larger than the negative control (NaCl) for any of the following allergens: Domestic mites (Dermatophagoides (D.)

pteronyssinus and *D. farinae*); German cockroach; dog, cat, and rabbit dander; birch, timothy (grass), and mugwort pollens; molds (*Cladosporium herbarium* and *Alternaria alternata*); egg whites; milk; peanuts; and codfish.(3)

### *Statistical Analyses*

Choice of lung function cut-offs at birth were based upon commonly used statistical approaches of median values, as well as previously identified clinical cut-off of  $t_{PTEF}/t_E < 0.20$ .(2)

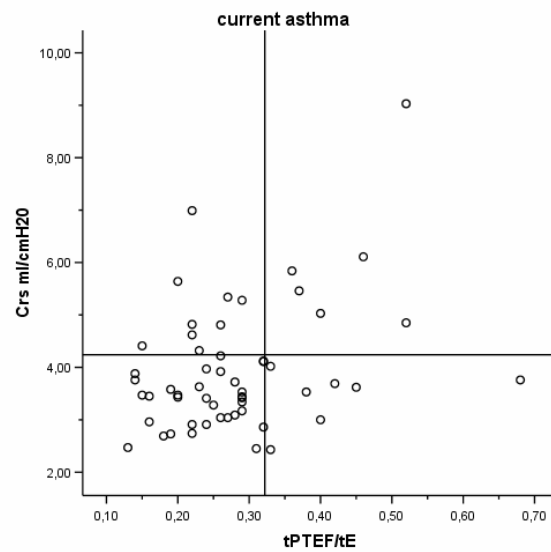
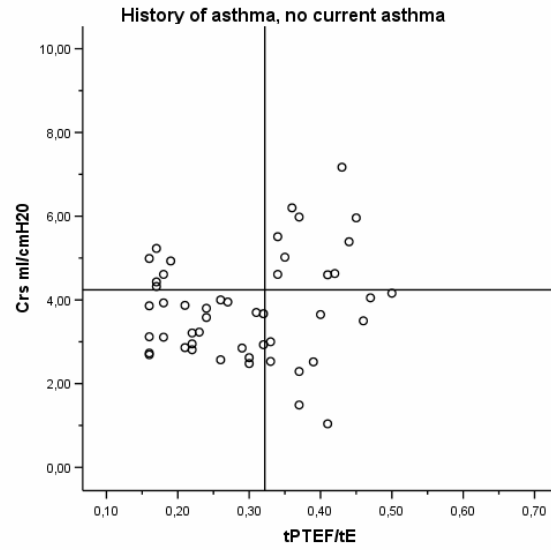
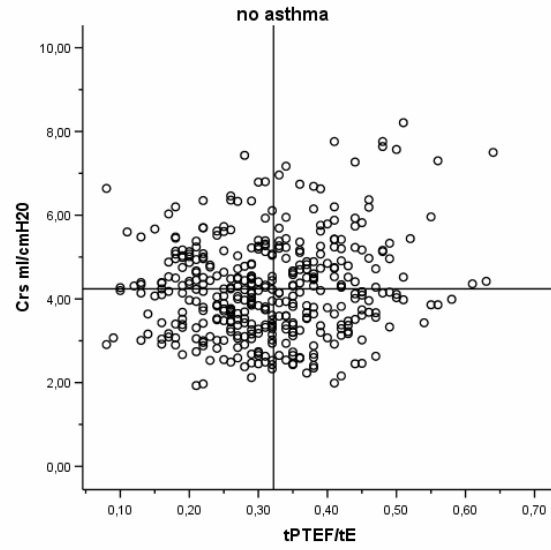
Initially, we analysed prevalence of asthma ever in combined quartiles of  $t_{PTEF}/t_E$  and Crs at birth, i.e. in 16 subgroups. The results indicated that subjects with Crs in the lowest two quartiles and  $t_{PTEF}/t_E$  in the lowest quartile had a much higher risk of asthma than the other subjects. Hence median was used as cut-off for Crs, and the limit between the 1<sup>st</sup> and 2<sup>nd</sup> quartile was used as cut-off for  $t_{PTEF}/t_E$ . However, since 0.20 had been suggested as a cut-off for  $t_{PTEF}/t_E$ , this value was later used as a cut-off in the analyses presented in the main paper. No such cut-off has been suggested for Crs, and hence median remained as the cut-off for Crs.

## **RESULTS**

Supplemental Figure 1 demonstrates raw data for correlations between Crs and  $t_{PTEF}/t_E$  (shortly after birth) in relation to clinical outcome at 10 years.

## **SUPPLEMENTAL FIGURE 1**

Lung function at birth measured by time to reach peak flow/total expiratory time ( $t_{\text{PTEF}}/t_{\text{E}}$ ) and compliance of the total respiratory system (Crs) ( $\text{ml}\cdot\text{cmH}_2\text{O}^{-1}\cdot\text{kg}$ ) in children with no asthma, a history of asthma without current asthma, and current asthma at ten years. The horizontal and vertical lines represent median values.



## REFERENCES

- (1) Nafstad P, Jaakkola JJ, Hagen JA, Botten G, Kongerud J. Breastfeeding, maternal smoking and lower respiratory tract infections. *Eur Respir J* 1996;9(12):2623-9.
- (2) Lødrup Carlsen KC, Carlsen KH, Nafstad P, Bakkevig L. Perinatal risk factors for recurrent wheeze in early life. *Pediatr Allergy Immunol* 1999;10(2):89-95.
- (3) Lødrup Carlsen KC, Håland G, Devulapalli CS, Munthe-Kaas M, Pettersen M, Granum B, Lovik M, Carlsen KH. Asthma in every fifth child in Oslo, Norway: a 10-year follow up of a birth cohort study. *Allergy* 2006;61(4):454-60.
- (4) Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *European Respiratory Journal — Supplement* 1993;16:5-40.
- (5) Zapletal A, Samanek M, Paul T. Lung function in children and adolescents. Methods, reference values. *Prog Respir Res* 1987;22:113-218.
- (6) Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing — 1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000;161(1):309-29.
- (7) Carlsen KH, Engh G, Mork M. Exercise-induced bronchoconstriction depends on exercise load. *Respir Med* 2000;94(8):750-5.