INTRODUCTION

Asthma is the most common chronic illness in childhood in industrialized countries, with prevalence reaching up to 20%.\textsuperscript{1,2} Research has identified familial, pre- and perinatal risk factors such as parental asthma, parental smoking, maternal pre-pregnancy obesity, and preterm birth to the later development of asthma in childhood.\textsuperscript{3,4} Maternal age at birth has been related to asthma in childhood. Some studies indicate that children born to younger mothers are at a higher risk of asthma, and it is unclear if this is a marker for other aspects such as socioeconomic status or lifestyle.\textsuperscript{4,5,6,7} Recently, advanced paternal age has been associated with higher risk of adverse pregnancy and offspring outcomes including e.g. spontaneous abortions\textsuperscript{8,9}, stillbirth\textsuperscript{10,11}, preterm birth\textsuperscript{12,13} and psychiatric diseases such as autism\textsuperscript{14,15} and schizophrenia\textsuperscript{16,17}. The biological mechanisms behind these associations are unknown, but may be related to age-related de novo mutations and epigenetic changes in male germ cells. The large number of cell divisions during spermatogenesis increase significantly with age resulting in an approximately 150 divisions by the age of 20 years and 840 divisions by the age of 50. Each time the cell divides, the replication of the genome introduces the possibility of mutations.\textsuperscript{18,19} Mutations inherited by the offspring can potentially lead to negative effects on their health. It has been suggested that epigenetic alterations caused by age-induced changes in the DNA methylation and chromatin structure can by structural inheritance be transmitted to offspring.\textsuperscript{20} Epigenetic studies of asthma have primary focused on DNA methylation, because of the close functional relationship between DNA methylation and gene expression. DNA methylation signatures in cord blood have been associated with increased risk of asthma during childhood (odds ratio=3.9).\textsuperscript{21} Thus, advanced paternal age may be related to risk of asthma in offspring, but this association has only been examined in a large Swedish register study with limited confounder information and outcome based on asthma diagnosed at hospital and prescription data.\textsuperscript{22} We will therefore examine the association between parental (both maternal and paternal) age at conception and the risk of development of parental reported doctor-diagnosed asthma in 7 years old children in a cohort study in Denmark taking various potential confounders like parental smoking and asthma into account.

MATERIALS AND METHODS

Design and study population
A cohort study which is going to be based on the population-based birth cohort, the Danish National Birth Cohort (DNBC) which enrolled 101,042 women in total from 1996 to 2002.\textsuperscript{23} Only singletons
and only one pregnancy per women (the first in the sampling period) will be included. Recruitment for the cohort was nationwide and performed by the general practitioners; and participation implied four telephone interview: during weeks 12-16 and weeks 30-35 of pregnancy, and when the child was 6 and 18 months old. Furthermore, during the month of the child’s 7th birthday, the parents were asked to fill in either a mailed or a web-based questionnaire. The interview in pregnancy week 12-16 comprised questions about risk factors for asthma (maternal parity and pregnancy BMI, parental socioeconomic factors, smoking and asthma). The questionnaire at age 7 dealt with lifestyle and health of the child including among others asthma. The cohort has been described in details elsewhere.24

Exposure
Data on the maternal age was obtained at the recruitment to the study, and the paternal age was obtained from the Danish Medical Birth Registry in the majority of cases.

Outcome
The information on asthma was based on questions from the 7-year follow-up. The questions mainly follow the International Study of Asthma and Allergies in Childhood.25 Doctor-diagnosed asthma ever was generated from the question: ‘Has your child ever been diagnosed with asthma by a doctor?’ Previously the specificity of the question of ‘physician-diagnosed asthma’ has been found to be 99% in a study based on 7- to 8-year-old schoolchildren.26 Two other questions were: ‘Has your child had wheezing or whistling in the chest in the last 12 months’ and ‘Has your child ever had asthma’.

Confounders
Information on the potential confounders according to prior knowledge from other studies on the risk factors of asthma in children will be obtained primarily from the information collected during the telephone interview at 12-16 weeks gestation. The potential confounders are; maternal parity and pre-pregnancy BMI, preterm birth, parental smoking, asthma and socioeconomic factors.

Statistical analyses
Incidence proportion of asthma in 7 years old children will be calculated by dividing the number of children with asthma recorded at the 7-year questionnaire with the total number of answered questionnaires at this age. We will estimate relative ratios (RR) and 95% confidence intervals using log-binomial regression models with maternal and paternal age as separate variables. Log-binomial
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Regression will be used because risk of asthma is expected to exceed 10% in our sample, and this method enables us to estimate the RR directly. The models will be performed with maternal and paternal age as categorized variables (into 5-years intervals, with the 25-29 years at the offspring’s birth as the reference group) adjusted for the above mentioned potential confounders in several steps. Correlation between maternal and paternal age will be examined to clarify if these variables should be mutually adjusted for each other in the multivariate analyses. The interactions between the categorized exposure variables and the potential confounders will be evaluated. All analyses will be performed in STATA 11.0 (STATA Corp., Texas, USA).

Power calculation
We expect a cohort with response to the internet questionnaire from 45,000 parents of 7 years old children. If we assume that RR for having a child with asthma as a function of parental age on 1.10 and with a prevalence of asthma of 20%, we have 100% power to detect this association.

Permission
Approvals from The Danish Data Protection Agency will be obtained.

REFERENCES