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NEW STUDY

# The Wheezing Illnesses Study Leidsche Rijn (WHISTLER): Rationale and design

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**Abstract.** The Wheezing Illnesses Study Leidsche Rijn (WHISTLER) was initiated in December 2001 as a single-centre prospective birth cohort study and will include a population-based sample of at least 2000 healthy newborns. The aims of WHISTLER are to investigate determinants for wheezing illnesses (including neonatal lung function, viral infections, asthma-susceptibility genes and endotoxin exposure) and to derive a comprehensive risk score, that is appropriate for use in primary health care and allows for efficient planning of early preventive strategies. Baseline examination includes a questionnaire evaluating known risk factors for wheezing illnesses; anthropometric measurements; measurements of infant and parental lung function; and sampling of infant and parental DNA. Participants will be followed for respiratory events using data from a daily respiratory symptom questionnaire; visits to the general practitioner (primary health care visits, drugs prescriptions and hospital referral); viral sampling during wheezing episodes; and house dust sampling. Based on actual neonatal care practice and embedded in a larger epidemiological study, the Utrecht Health Project, WHISTLER will provide an unique framework to address issues in childhood respiratory disease that are currently insufficiently understood. In particular, WHISTLER will provide a well-balanced view on the prognostic power of neonatal lung function and genetic and environmental factors (including viral infections and endotoxin exposure) to predict wheezing illnesses from birth to young adulthood and beyond. In the scope of prevention, WHISTLER is expected to support the design of solid based prevention measures to reduce respiratory morbidity, mortality and associated costs, and to improve quality of life.

**Key words:** Birth cohort, Etiology, Infants, Lung function, Wheezing

## Introduction

Wheezing illnesses are the most common cause of morbidity and mortality in infancy and childhood and have a large impact on health care [1, 2]. The prevalence has increased substantially over the last few decades [3, 4]. Of equal importance is the growing evidence for a link between childhood wheezing illnesses and chronic respiratory disease in adult life [5, 6]. For effective targeting of prevention strategies more insight in risk factors for different wheezing phenotypes is necessary [7]. Moreover, such strategies should probably be implemented as early in life as possible. Unfortunately, to our knowledge, studies specifically aiming at the identification of the high-risk infant in primary care at a very early age are not available. The Wheezing Illnesses Study Leidsche Rijn (WHISTLER) is designed firstly, to investigate determinants of different wheezing phenotypes and secondly, to derive a comprehensive risk score for wheezing illnesses, that is appropriate for use in primary health care and allows for efficient planning of early preventive strategies. This paper describes the rationale and design of WHISTLER.

## Rationale

The prevalence of wheezing in Western Societies has doubled over the last 20 years [3, 4]. Recent data on British pre-school children show that 29% of all children have a history of ever wheezing, 26% are current wheezers, 19% have been diagnosed to have asthma and 26% are treated for wheeze [3]. The rising prevalence around the world imposes a growing burden on society in terms of consultations in primary care, hospital admissions, associated health care costs and quality of life of affected children and their families [2, 8]. It is estimated that preschool wheeze in the UK costs the health service a total of 53 million UK pounds. The largest expenditure, 34 million UK pounds, is for primary care, followed

by prescriptions costs representing 11 million UK pounds [2]. In addition, wheezing illnesses in early childhood predispose to chronic respiratory disease in later life, which requires long-term care with additional financial and social impact [5, 6, 9].

A number of prospective studies has contributed to the identification of risk factors for wheezing illnesses, including birth cohort studies such as the Tucson Children's Respiratory Study [10], the Multi Allergy Study (MAS) [11] and the Prevention and Incidence of Asthma and Mite Allergy Study (PIAMA) [12]. Foetal growth, male gender, neonatal lung function, maternal age, breastfeeding, family history of asthma or atopy, day care attendance, number of siblings, tobacco smoke exposure, air pollution, socio-economic status and pet keeping are associated with wheezing illnesses [13–20]. Unfortunately, easily applicable scores to reliably estimate the risk to develop wheezing illnesses in healthy newborns are not available [21]. Such risk scores are a prerequisite to target prevention measures and early interventions. More general measures such as avoidance of tobacco smoke and allergen exposure are already advocated in very young children [22–24]. However, many other preventive actions require more individually tailored risk estimations. WHISTLER will combine the prognostic value of different risk factors for wheezing illnesses and aim at developing a prediction rule. This model should be an easy applicable algorithm that will support the physician to detect high risk children in daily practice. Apart from established risk factors, WHISTLER will pay specific attention to other putative determinants, such as impaired neonatal lung function, asthma-susceptibility genes, viral infections and endotoxin exposure.

There is ample evidence that a relatively small airway calibre contributes to the risk of wheezing illnesses [14, 25]. Impaired foetal or neonatal lung growth might lead to smaller airway calibre and consequently to an increased risk for wheezing during viral infections or asthmatic inflammation. Only a few small prospective cohort studies investigated premorbid lung function in association with subsequent respiratory illnesses [14, 25–28]. However, because neonatal lung function testing is technically difficult and often requires sedation, sufficient sample size is difficult to obtain and results are conflicting. Some authors found a relationship between lung function and subsequent wheezing [14, 25, 26], while others did not [27, 28]. Non-invasive techniques for neonatal lung function measurement have recently been developed to assess compliance and resistance of the respiratory system under normal breathing conditions. These techniques rely on the relaxation of respiratory musculature after a brief airway occlusion during expiration (Hering-Breuer reflex) and primarily reflect the mechanical properties of the lung and airways [29–31]. These techniques are rapid and simple to perform by experienced staff during the baby's natural sleep. International guidelines are available to ensure safety, precision and reproducibility [32, 33]. WHISTLER aims to elucidate the relation between neonatal lung function and subsequent wheezing illnesses in a large population-based cohort using this relatively new technique. Notably, measurement of passive airway mechanics seems particularly suitable for predictive purposes in a general population of newborns. WHISTLER will investigate the possibility to use neonatal lung function as an additional predictive parameter for wheezing illnesses in routine daily practice.

A relatively new area is the search for susceptibility variants of genes for wheezing and asthma that may, in interaction with environmental factors, play a role in development of disease [34]. Different genomic regions that contain so-called "atopy/asthma genes" have been identified. Identification of susceptibility genes in these regions and knowledge about gene-gene and gene-environment interactions will increase our understanding of the genetic basis of wheezing and asthma, which may be crucial for the development of primary prevention strategies. The role of viral infections in the inception of asthma remains controversial. Early exposure to certain viral respiratory infections (e.g., hepatitis A) may protect against wheezing illnesses, whereas some other infections (e.g., respiratory syncytial virus) may exert the opposite effect [35, 36]. The relation between neonatal lung function, wheezing and specific viral infections is not known. In addition, the exposure to endotoxin and other bacterial wall components, found in many indoor and outdoor environments, is important to investigate. This may play a role in the development of tolerance to allergens found in natural environments and consequently in the development of atopic diseases [37].

The need to further investigate determinants of wheezing illnesses and to derive comprehensive and practical prediction rules to identify high risk infants at a very early age provide a compelling rationale for the WHISTLER. Based on actual neonatal health care practice and embedded in a larger epidemiological study as described hereafter, the evolving WHISTLER-data will provide a unique framework to address issues in childhood respiratory disease that are currently insufficiently understood. In particular, WHISTLER will provide a well-balanced view on the prognostic power of neonatal lung function and genetic and environmental factors to predict wheezing illnesses from birth to young adulthood and beyond. WHISTLER is expected to support the design of solid based prevention measures to reduce morbidity, mortality and associated costs, and to improve quality of life.

## Study objectives

The aim of WHISTLER is two-fold: (1) to investigate putative new etiologic determinants of wheezing

illnesses with particular focus on neonatal lung function, asthma-susceptibility genes, viral infections and endotoxin exposure; (2) to develop a risk score for the occurrence of wheezing illnesses from childhood to adulthood using determinants that are currently available in primary neonatal care. Relatively new measures that are currently not available to primary care, such as neonatal lung function and susceptibility genes, are studied for additional predictive power before considering practical implementation.

## Study design

WHISTLER is a single-centre, prospective birth cohort study and will include a population-based sample of at least 2000 healthy newborns. Recruitment and examination of infants started in December 2001. All individuals will be followed for respiratory events from birth until young adulthood and beyond. Written informed consent is obtained from parents. The paediatric medical ethics committee of the University Medical Centre Utrecht has approved the protocol.

## Study population

### *Setting*

Leidsche Rijn is a new residential area under construction near the city of Utrecht in the Netherlands, in which 80,000 to 100,000 people of various ages, social, cultural and economic backgrounds will have settled by the year 2015. A birth rate of 100/2500 inhabitants/year will lead to an expected 400 births in 2004 and at least similar numbers over the next years. In the future there will be seven primary health care centres, each eventually serving 10,000 to 12,000 inhabitants. Each centre will have approximately five general practitioners with supporting staff and a pharmacist. Every two centres will have their own well-baby health care facility, an obstetric practice and a first aid service. This infrastructure provides an excellent basis for epidemiological research. In 2000, a large health monitoring study was initiated in Leidsche Rijn, the Utrecht Health Project (Dutch acronym LRGP: Leidsche Rijn Gezondheids Project, www.lrgp.nl). This study aims to generate valuable data from all inhabitants on determinants of health and disease and on the use and efficiency of innovative healthcare. Inhabitants are invited to participate in the study when they register with their new general practitioner. Written informed consent is obtained from all participants. During an extended first consultation an "Individual Health Profile" (IHP) is compiled that provides an update on the health status of the participant and contains information that is relevant for the physician as well as for research purposes. This IHP comprises the following components: an update on medical history and pharmacotherapy; questionnaires on socio-economic status, mental health, cardiovascular risk, trauma, dietary intake, life-style factors (e.g., smoking, alcohol) and housing; and biometry (age, gender, weight, length, blood pressure, blood sample for total cholesterol, HDL/LDL cholesterol and glucose, 12-lead electrocardiogram and pulmonary function). Medical history as well as follow-up (including drug prescriptions, additional examinations and referral to other primary care staff or to secondary care) is registered in the patient's electronic database (Medicom®, PharmaPartners, the Netherlands) by the general practitioners during routine health care using the International Classification of Primary Care (ICPC) [38]. The database of the Utrecht Health Project is updated with this encoded follow-up information every 3 months.

### *Recruitment WHISTLER*

In the Netherlands parents must register a new birth at the city council within 3 days. The council notifies WHISTLER and "Stichting Thuiszorg", the organization dedicated to well-baby care until the age of 4 years. A nurse of this institution routinely visits newborns at home in the first week after birth for their neonatal heel-prick test. During this visit the nurse provides an information brochure of WHISTLER to the parents. Within 14 days the WHISTLER staff contacts households by telephone and eligible children are invited to participate in the study. Exclusion criteria are parents not participating in the Utrecht Health Project, gestational age $<36$ weeks, major congenital abnormalities and neonatal respiratory disease. A record is kept of all newborns with exclusion criteria and of those not consenting to participate.

## Baseline examination

Figure 1 gives an overview of enrolment and data collection. Parents and child(ren) are invited to the ambulatory clinic of the Utrecht Health Project in the second or third week of life before any respiratory illness is present. Table 1 provides a list of the measured components of the risk profile at baseline examination.

### *Questionnaires*

Information on the child is gathered by a questionnaire filled in by the mother during baseline examination with regard to pre-, peri- and postnatal factors (Table 1). Parental data are obtained from the IHP. WHISTLER primarily focuses on general

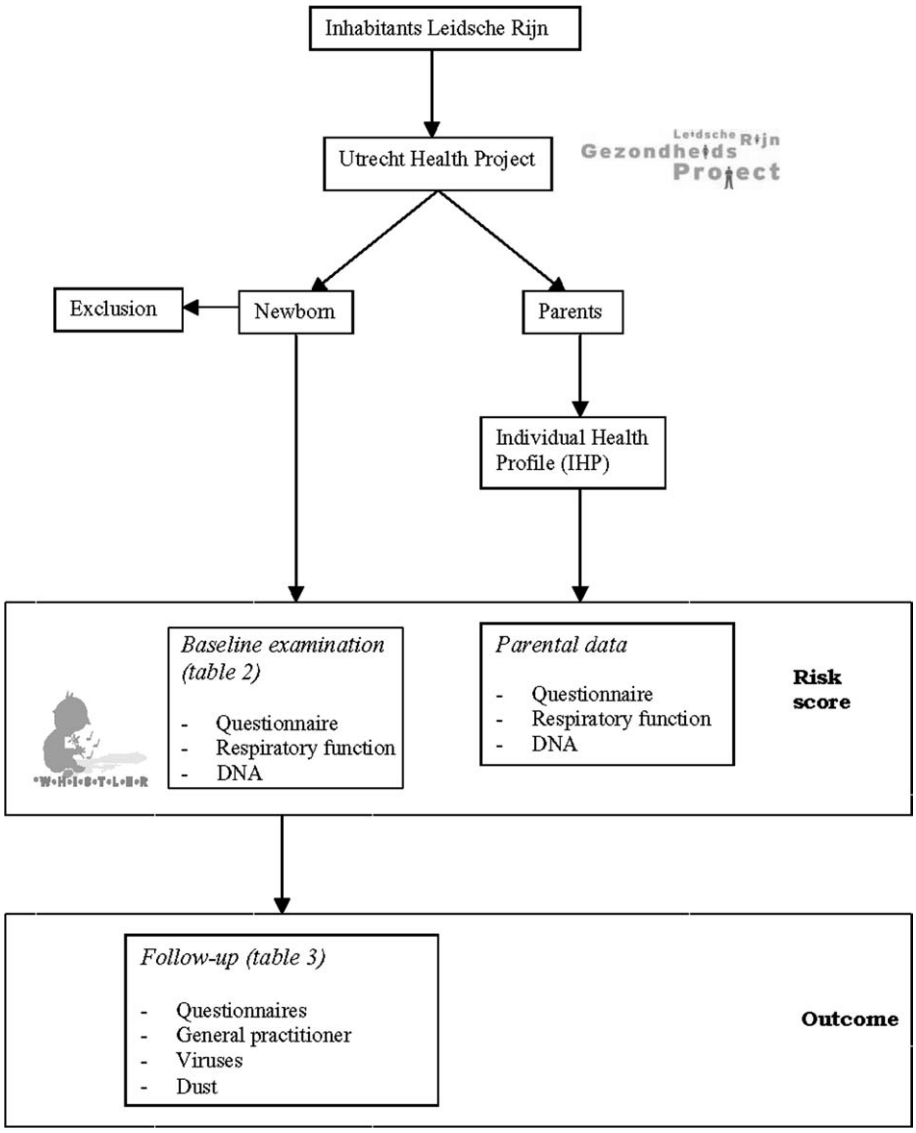


**Figure 1.** Enrolment and data collection.

characteristics, history of asthma and atopy, dietary intake, lifestyle factors, medication use and housing.

*Antropometric measurements*

Body weight is measured using a standard electronic scale and body length using an infant stadiometer. Maximal occipital-frontal and thoracic circumference are assessed using a tape measure.

*Respiratory function*

Respiratory function is measured in all infants at the baseline examination prior to any upper or lower respiratory illness. Measurements are performed during natural sleep without use of any sedation and with the infant in supine position. The resistance ($R_{rs}$), compliance ($C_{rs}$) and time constant ($t_{rs}$) of the total respiratory system are measured using the single breath occlusion technique (SBT). Briefly, end-inspiratory airway occlusion induces a Hering-Breuer reflex with complete relaxation of respiratory muscles and passive expiration after release of the occlusion. During periods of no flow, rapid pressure equilibration can be reached. The pressure at airway opening ($P_{ao}$) represents the elastic recoil pressure of the respiratory system and can be related to changes in volume and flow in order to calculate the compliance and resistance of the respiratory system. The slope of the descending portion of the passive flow-volume loop represents the expiratory time constant of the respiratory system [30, 31]. Flow is measured using a heated Lilly-type pneumotachimeter (series 8300, linear range 0–10 l/min; Hans Rudolph Inc., Kansas City, MO, USA) attached to a face mask sealed with silicone putty (Magic Putty, medium, Oldelft Benelux BV, Delft, The Netherlands) to prevent air leaks and to minimize dead space (Figure 2). Volume is obtained as the electronic integration of flow. Pressure inside the facemask is measured with a pressure

**Table 1.** Components of baseline examination

| |
|---|
| *Questionnaire* |
| Prenatal data |
| General characteristics (gestational age, gender) |
| Maternal smoking habits |
| Exposure to pets |
| Occurrence of maternal viral infections |
| Maternal probiotic use |
| Perinatal data |
| Birth weight and length |
| Feeding history |
| Breast or bottle feeding |
| Infants feeding formulas |
| Parental data (Utrecht Health Project) |
| General characteristics (age, gender, ethnicity, socio-economic status) |
| History of asthma or atopy |
| Dietary intake |
| Life-style factors (smoking, alcohol) |
| Medication use |
| *Antropometric measurements* |
| Weight and length |
| Maximal occipital-frontal and thoracic circumference |
| *Respiratory Function* |
| Infant |
| Respiratory resistance (Rrs) |
| Respiratory compliance (Crs) |
| Respiratory time constant (trs) |
| Parents |
| Forced expiratory volume in the first second (FEV1) |
| Forced vital capacity (FVC) |
| Peak expiratory flow (PEF) |
| Forced expiratory flow at 50% and 75% of FVC (FEF50, FEF75) |
| *DNA sampling* |
| Infant and parental DNA |

transducer (Honeywell, type 163PC01D75, Morristown, NJ). Flow, volume and pressure at the infants' mouth are recorded and presented on a monitor. Brief occlusions are performed manually close to maximal inspiration. The occluded airway opening is released when a plateau in mouth pressure is reached. Measurements are done according to the guidelines of the European Respiratory Society with a smooth expiration within 10% of the previous expiration and without evidence of glottic closure, braking or active expiratory effort, duration of a pressure plateau $\geqslant$100 ms and variability $<10$ Pa and linearity of the descending part of the passive flow-volume loop over at least 40% of expiration with $r^2 > 0.99$ [33]. At least three technically acceptable flow-volume curves are required to calculate mean resistance, compliance and time constant. Lung function measurements are performed by WHISTLER staff. Elaborate inter-rater reliability studies are performed as part of WHISTLER.

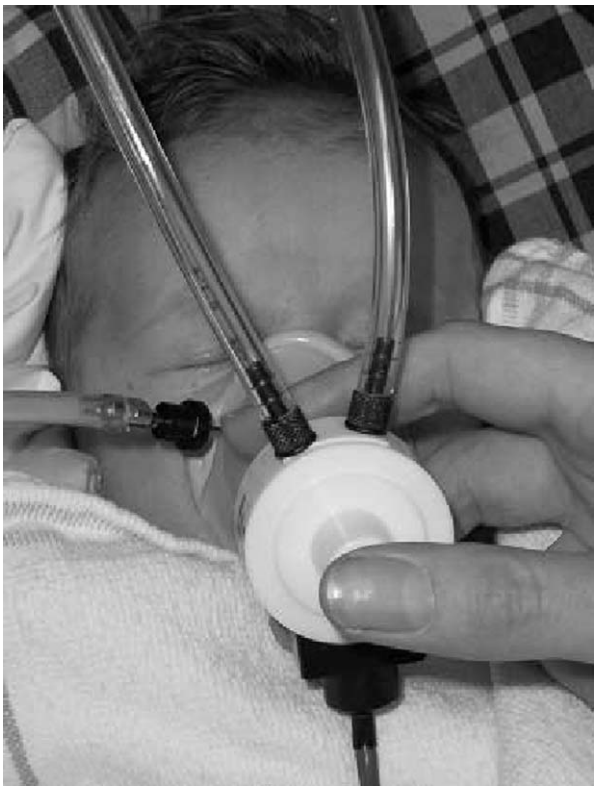

**Figure 2.** Neonatal lung function measurement.

Parental respiratory function is measured as part of the IHP using spirometry according the guidelines of the American Thoracic Society (Table 1) [39].

*DNA sampling*

Genomic DNA is extracted from buccal cells of infants and parents that has high quality and allows for a large number of PCR assays from a single sample [40]. Parents are instructed not to eat or drink for half an hour before sampling. Per person 3 buccal swabs (Catch-all Sample Collections Swabs, QEC091H, Epicentre, Madison, USA) are collected. DNA is extracted using the QIAamp DNA blood mini kit (Qiagen) and concentration is determined using PicoGreen (Molecular Probes). DNA is stored in a $-20$ °C freezer until polymerase chain reaction (PCR) amplification and genotyping will be performed.

## Follow-up and study end-points

Comprehensive follow-up for wheezing illnesses is achieved in different ways (Table 2). During the first year of life all parents fill in daily questionnaires with regard to wheeze, cough and fever of their babies. New questionnaires and reinforcements are sent on a monthly basis to the parents. If parents still fail to return the questionnaire, they are contacted by telephone. Only 9 out of 281 (3.2%) infants have been lost to follow-up since the

**Table 2**. Study end points

| |
|---|
| Parentally reported wheeze during the first year of life |
| Airway infections during the first year of life |
| Physician diagnosed wheezing illnesses in primary care (ICPC) |
| Cough |
| Upper respiratory infection |
| Acute laryngitis/tracheitis/croup |
| Acute bronchitis/ bronchiolitis |
| Influenza |
| Pneumonia |
| Asthma |
| Chronic obstructive pulmonary disease |
| Respiratory problems associated referral to pediatric hospitals |
| Wheezing associated mortality |

start of the study. Alongside daily recording of respiratory complaints, the infants exposure to smoke and pets, feeding habits, day care attendance, signs and symptoms of allergy and growth during the first year of life are recorded through this questionnaire. Information from the general practitioner including data on primary health care visits, drug prescriptions, hospitalisations and intensive care admissions for wheezing illnesses, are obtained from the database of the Utrecht Health Project as described above.

To relate specific viral respiratory infections to specific wheezing episodes, naso-pharyngeal swabs are taken in a subgroup of infants at the second day of a parent-reported wheezing episode. After receiving a precise instruction at baseline examination, parents collect the samples by gently rubbing respectively, the throat and one of the nostrils using a swab. After sampling, swabs are collected in a virus transport (GLY) medium and sent by the parents to our laboratory the same day, where they are stored at −80 °C until further analysis (Reverse transcriptase) polymerase chain reaction assays ((RT)-PCR) for rhinovirus and enterovirus, influenza A and B, respiratory syncytial virus (RSV), human metapneumovirus (HMPV), coronavirus 229E and OC43, adenovirus, chlamydia pneumoniae and mycoplasma pneumoniae are performed.

House dust sampling is performed by a field technician at some point during the first year of life. A home visit is made to collect dust from the living room and the infant's and parent's mattresses by vacuuming part of the surface area for 2 min/$m^2$ as described before [41, 42]. Dust is collected in preweighed sample tubes (Greiner, polypropylene 50 ml; no. 210296) on special sample filters (70 mm glass fibre filter; Schneider & Schuell, ref. no. 370104), which are held in an ALK filter holder during sampling. The dust weight is calculated as the difference between the post- and pre-weight of the sample tube. Until weighing, extraction and further analysis, sample tubes are stored in a freezer at −20 °C. The dust is extracted and various specific biologic agents such as allergens and bacterial endotoxins, are determined.

The endpoints of WHISTLER are wheezing illnesses assessed at different levels (parents reported wheeze, viral wheeze, and physician-diagnosed wheeze). Parents reported wheeze is assessed on a daily basis using a questionnaire and viral sampling is performed in a subset of infants during parents reported wheezing episodes. Physician-diagnosed wheeze is assessed using different categories of wheezing illnesses in primary care, according to the International Classification of Primary Care (ICPC). Additionally, hospitalisations and mortality due to wheezing illnesses are studied. These endpoints enable the investigation of different aspects of wheezing illnesses. Table 2 provides a list of endpoints assessed in WHISTLER.

## Sample size and data analysis

The birth rate in Leidsche Rijn is estimated at 100/2500 inhabitants per year, leading to an expected 400 births in 2004 and at least similar numbers over the next years. As WHISTLER aims to ultimately cover all participants of Leidsche Rijn the inclusion is intended to continue throughout the expanding phase of the district until an expected 2000 newborns are measured. Given a non-response of around 25%, this number will be achieved in 6–8 study years. The association between lung function parameters and wheezing illness will be analysed using logistic regression techniques. An estimated 20–30% of children will attract some form of (first) wheezing illness that requires medical attention of a general practitioner [3]. This will enable multivariate prognostic modelling with many variables. All available prognostic variables will first be univariately evaluated with respect to outcome (wheezing illnesses or not). Subsequently, a multivariate model will be constructed from all univariately relevant variables except lung function. Internal validity of models will be evaluated using bootstrapping methods. The additional predictive capacity of lung function parameters will be evaluated by adding these to the first model. Receiver Operating Characteristic curves of different models will be compared (Hosmer–Lemeshow). These procedures will be repeated within certain risk groups of children.

## Data management

The Data Coordinating Centre is located at the Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, and is responsible for

data entry of both WHISTLER and the Utrecht Health Project. Data of WHISTLER collected at baseline as well as the daily questionnaires are entered on Teleform (Cardiff Software Inc., Vista, CA), an optical recognition-based technology that scans data collection paper forms and exports data to a computer database. The data entry software interprets data, which are verified by an operator. After data entry is complete, all data pass through an extensive editing process to check for inconsistencies at the coordinating centre. Lung function tests are analysed by the researchers and data are sent to the data coordinating centre. Subsequently, WHISTLER data are linked to parental data collected in the Utrecht Health Project. The conduct of this study, including data acquisition, data analysis and reporting is the responsibility of the researchers.

## Limitations

A major prerequisite for applicability of prediction rules in general practice is that the measurement of predictors is easy for both subjects and health care. This limits the use of more invasive measurements, e.g., taking blood samples for immunologic tests. In addition, WHISTLER aims to include a very large sample of the newborns in Leidsche Rijn, which limits the use of invasive tests as the participation rate would decline considerably. More complex interactions (including immunologic factors) that may influence the development of wheezing illnesses can therefore not be studied in the design of WHISTLER as presented here. WHISTLER intends to examine these issues in subgroups of infants (''nested designs'').

## Current status of the study

At the time of writing 350 of 463 (75.6%) eligible infants have consented to participate in WHISTLER. In 281 (80.4%) infants lung function measurement has been performed successfully. The principal reason for unsuccessful lung function measurement is an awake state of the child.

## Conclusion

Wheezing associated illnesses occur frequently in infancy and childhood and may predispose for adult chronic respiratory disease. Development of preventive strategies is a high public health priority. Through its unique design and embedding in the Utrecht Health Project, the WHISTLER will provide new information on determinants of wheezing illnesses and prediction of the occurrence of different wheezing phenotypes in childhood and adult life. It is envisioned that these wheezing illnesses prediction rules will be applicable and used in clinical practice to improve identification of subjects at risk allowing more effective prevention measures in defined high risk groups and subsequently reduce morbidity, mortality and associated costs and to improve quality of life.

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