

**STUDENT NAME:** *Maggie Ong*

**PROJECT TITLE:** A longitudinal study on the value of the methacholine challenge test as a diagnostic aid for asthma in high-risk adolescents

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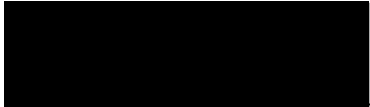
**SUMMARY:**

The objective of this study was to investigate the utility of the methacholine challenge test as a measure of airway hyperresponsiveness, in aiding the clinical diagnosis of asthma. The study population comprised 330 high-risk 15-year-olds who have been followed since birth as a part of CAPPS, a multifaceted intervention trial on asthma prevention. Interventions consisting of HDM, pet, and ETS avoidance, maternal and infant dietary modifications, and daycare avoidance were implemented over the first year of life. Assessments at 15 years of age included a physician examination, methacholine challenge test, and skin prick test. ROC curves were constructed from sensitivities and specificities calculated for PC<sub>20</sub> cut points from 1.0 to 8.0 mg/mL, using physician diagnosis as the gold standard. AUC's were calculated using the trapezoidal rule and the DeLong method. An AUC of 0.73 was measured for asthma overall, which indicates that the methacholine challenge is a fair test (95% CI 0.65-0.82). This is an improvement from the value obtained at 7 years of age. When stratified by sex and atopy, an AUC of 0.50 was measured for adolescent females with non-atopic asthma, which describes it as a failure. The best PC<sub>20</sub> cut point defining AHR in asthma overall was  $\leq 5.5$  mg/mL (sensitivity of 61.0% and specificity of 85.2%), which is an increase from the value at 7 years of age. These findings show that the methacholine challenge test is useful in assisting the diagnosis of asthma overall in high-risk adolescents, but not of non-atopic asthma in adolescent females.

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## **Introduction**

Asthma is one of the most common chronic diseases in adolescence as an estimated 11.8% of Canadian adolescents are affected<sup>1</sup>. It has a major impact on morbidity with 54.9% of adolescents reporting episodic attacks<sup>2</sup>, and causes an average of 20 pediatric deaths each year<sup>3</sup>. Furthermore, asthma poses significant economic burden as \$12 billion was spent on treating chronic respiratory diseases including asthma in 2010<sup>4</sup>. It is also the leading cause for absenteeism from school, and parents missing work<sup>5</sup>. Given its epidemic nature, there is a great need for the accurate diagnosis of asthma.

A reliable diagnosis is difficult to obtain in childhood because asthma is a heterogeneous disease with broad clinical expression and a lack of specificity for its symptoms. There are three main wheezing phenotypes in childhood, but not all are indicative of asthma. These phenotypes vary in age of onset, triggers, severity, treatment, and pattern of remission and relapse. They include transient early wheezing, persistent early-onset wheezing, and late onset wheezing<sup>6</sup>. Transient early wheezing is associated with prematurity and smoking, but is usually outgrown in the first 3 years of life. Persistent early-onset wheezing involves recurrent episodes of wheeze usually of viral origin that persist into adolescence. These children however have no atopy. Atopic wheezing, often associated with eczema and airway pathology, is the only one indicative of asthma, and tends to persist beyond adolescence.

While the diagnosis of asthma is less difficult to make in adolescents than in younger children as symptoms are more specific in this age group<sup>6</sup>, other problems still exist. Studies show that many adolescents relapse following an asymptomatic period during which they seem to have outgrown their asthma<sup>7</sup>. Furthermore, in some of those who remain asymptomatic, airway hyperresponsiveness (AHR) continues to persist<sup>8-9</sup>. The diagnosis of asthma is currently a clinical one based on a history and physical examination by a physician, and is often assisted by the use of pulmonary function tests<sup>6</sup>. The methacholine challenge is an objective test that has been commonly used to measure airway responsiveness. Airway hyperresponsiveness is a cardinal feature of asthma.

AHR is a phenomenon occurring in most asthmatics in which the airways constrict reversibly to a greater degree in response to physical or chemical stimuli in the environment<sup>6</sup>. Airway responsiveness is commonly measured by the methacholine challenge test in which increasing concentrations of methacholine are used to provoke airway constriction. The measurement used in the methacholine challenge test is the PC<sub>20</sub>, which is defined as the provocative concentration of methacholine at which the forced expiratory volume in one second (FEV<sub>1</sub>) falls by 20% from baseline. AHR is present when the PC<sub>20</sub> is below a defined cut point. A PC<sub>20</sub> of 8.0 mg/mL or less is commonly used to define AHR in adults<sup>10</sup>. In school-aged children, our research group and others have demonstrated that the cut point to define AHR is lower (i.e. less methacholine is required to decrease FEV<sub>1</sub> by 20%)<sup>11-14</sup>.

We studied the diagnostic value of the methacholine challenge test in high-risk adolescents at 15 years of age from the Canadian Asthma Primary Prevention Study (CAPPS). CAPPS is a longitudinal randomized controlled trial that was initiated in 1994 to determine the effectiveness of a multifaceted intervention program in preventing asthma in high-risk children<sup>15</sup>. They are

**STUDENT NAME:** *Maggie Ong*

high-risk because they have a strong family history of asthma or atopic disease. The study is unique in that the intervention program is multifaceted, so it is more likely to succeed than studies that use a monofaceted approach<sup>16</sup>. The interventions consist of reduced house dust mite (HDM), pet, and environmental tobacco smoke (ETS) exposures, exclusive breastfeeding for the first 4 months, delayed introduction of solids until 6 months, maternal and infant dietary limitations, and avoidance of daycare. These measures were applied from the third trimester through the first year of life, a period widely considered a window of opportunity for modifying asthma risk<sup>16</sup>. Prior publications from the study showed significant reductions in asthma at 1, 2, and 7 years of age<sup>17-19</sup>. The assessments were recently completed in the cohort at 15 years of age.

The utility of the methacholine challenge test was previously assessed in high-risk children at 7 years of age from CAPPS, using physician diagnosis as the gold standard to which the test was compared<sup>13</sup>. The optimal PC<sub>20</sub> cut point conferring the greatest sensitivity plus specificity for AHR was  $\leq 3.0$  mg/mL (sensitivity of 80.0% and specificity of 49.1%). On the other hand, the balanced PC<sub>20</sub> cut point where sensitivity and specificity are most equal was  $\leq 2.0$  mg/mL (sensitivity of 63.1% and specificity of 64.7%). The methacholine challenge was determined to be a fair diagnostic test in 7-year-olds of this cohort with an AUC of 0.699. Our interest to determine the change in AUC, sensitivity and specificity of the test, and the best PC<sub>20</sub> cut point in this cohort from 7 to 15 years.

Similar analyses were done in the Study of Asthma Genes and the Environment (SAGE), which is a longitudinal population-based birth cohort study initiated in 1995, consisting of low and high-risk children living in urban and rural Manitoba<sup>11</sup>. Within the birth cohort is nested a case-control study in which specified children were closely followed with respect to their asthma status, genotype, and environmental exposures. The diagnostic value of the methacholine challenge test was analyzed in the cohort separately at 7-10 and 11-14 years of age. At 7-10 years of age, the best PC<sub>20</sub> cut point defining AHR in asthmatics was  $\leq 4.0$  mg/mL (sensitivity of 65.4% and specificity of 64.6%)<sup>11</sup>. The AUC was 0.70 making the methacholine challenge a fair diagnostic test for asthma in children at 7-10 years of age from this cohort. On the other hand, at 11-14 years of age, the same values were calculated and found to be slightly different. Although the best PC<sub>20</sub> cut point remained unchanged at  $\leq 4.0$  mg/mL, the sensitivity and specificity increased with respective values of 71.3% and 79.3%<sup>12</sup>. Similarly, the AUC was higher at 0.79.

To summarize, the main objective of this project was to assess the utility of the methacholine challenge test in a population of high-risk adolescents at 15 years of age. We predict that the value of the test will improve as demonstrated by an increase in the AUC, and sensitivity and specificity of the test. We also expect that the best PC<sub>20</sub> cut point defining AHR in this cohort at 15 years of age will increase from the cut point at 7 years of age.

## **Materials and Methods**

The study population consists of 330 high-risk 15-year-old adolescents from Winnipeg and Vancouver who have been followed since birth as a part of the Canadian Asthma Primary Prevention Study (CAPPS)<sup>15</sup>. They are high-risk because they have at least 1 first-degree relative with asthma, or 2 first-degree relatives with other IgE-mediated allergic diseases, such as atopy, allergic rhinitis, and atopic dermatitis. When the study began, there were 545 families enrolled,

**STUDENT NAME:** *Maggie Ong*

with 281 infants randomized to the intervention group and 268 to the control group. By 15 years of age, 330 adolescents, 180 from the intervention group and 150 from the control group, returned to be assessed. Figure 1 describes the cohort in terms of retention numbers at each wave of the study since its initiation.

A multifaceted intervention program was applied from the third trimester through the first year of life<sup>15</sup>. The interventions consisted of the following: 1) Reduction of HDM exposure through the use of mattress covers, weekly washing of bedding, and application of benzyl benzoate to bedroom carpets and upholstered furniture in the most commonly used room; 2) Pet avoidance which involved the removal of cats and dogs from the home, or at least keeping them away from the child's bedroom; 3) A smoke-free environment as parents were counselled on smoking cessation; 4) Nutritional modifications such as exclusive breastfeeding in the first 4 months, delayed introduction of solids until 6 months, and exclusion of peanuts and seafood from maternal diet during pregnancy and from the infant's diet up until the first year of life. Cow's milk was also excluded from the infant's diet; 5) Avoidance of daycares. These measures were reinforced by the research nurse. The children were formally assessed at 1, 2, and 7 years of age.

The most recent assessments were completed over the past two years on the cohort at 15 years of age, with an important component being the physician assessment. The adolescents were assessed by asthma-expert physicians who were blinded to group allocation, and the methacholine challenge and skin prick test results. The physicians conducted structured interviews in which they enquired about cough, wheeze, and dyspnea with or without colds, as triggered nocturnally with wakening, during exercise, or laughing. The frequency of colds and use of bronchodilators and corticosteroids were also noted. Furthermore, a chest exam was performed to check for hyperinflation, Harrison's sulcus, wheeze, prolonged expiration, and decreased breath sounds, in addition to head and neck and dermatological exams. The goal was to determine if the adolescents had clinical diagnoses of asthma, allergic rhinitis, atopic dermatitis, or food allergies.

In addition to physician assessment, the adolescents underwent the methacholine challenge test according to Cockcroft's tidal breathing method which involves breathing quietly for 2 minutes while inhaling from a nebulizer<sup>10</sup>. A Wright nebulizer, calibrated for an output of 0.13–0.44 ml/min, was used to dispense the methacholine, while the Puritan-Bennett Renaissance II spirometer was used to measure lung function. Baseline and saline measurements were obtained prior to beginning the test, and adolescents with a baseline FEV<sub>1</sub> above 70% of the predicted value were safe to proceed. Most adolescents were started at a concentration of 1.0 mg/mL, with the concentrations being doubled over time to 8.0 mg/mL. Spirometry was performed after each concentration, and those who had a 20% fall in FEV<sub>1</sub> from baseline before reaching 8.0 mg/mL had the test stopped. At the end, all adolescents were given two puffs of salbutamol, followed by spirometry after 15 minutes to ensure their FEV<sub>1</sub> returned to near baseline and to assess for reversibility. The PC<sub>20</sub>'s were calculated from the following formula<sup>20</sup>:

$$\log PC_{20} = \log C_1 + \frac{(\log C_2 - \log C_1)(20 - R_1)}{(R_2 - R_1)}$$

**STUDENT NAME:** *Maggie Ong*

Where  $C_1$  is the second to last concentration of methacholine,  $R_1$  is the percent  $FEV_1$  reduction produced by  $C_1$ ,  $C_2$  is the last concentration causing a 20% drop in  $FEV_1$  from baseline, and  $R_2$  is the percent  $FEV_1$  reduction produced by  $C_2$ . The methacholine challenge tests were performed by a trained research assistant.

Skin prick testing was also done, according to the epicutaneous method, for sensitization to common environmental and food allergens. These included *Alternaria*, *Cladosporium*, *Penicillium*, HDM (*Dermatophagoides pteronyssinus* and *Dermatophagoides farina*), cockroach, egg white, soybean, cow's milk, trees, grass, weeds, ragweed, cat, dog, feathers, wheat, peanuts, and tree nuts, if indicated. Histamine 1.0 mg/mL and saline were used as positive and negative controls respectively. A positive test indicating atopy was a wheal with a mean diameter 3 mm greater than the negative control, 20 minutes after pricking the skin with a prick lanceter dipped in allergen solution. Skin prick testing was carried out by a trained research assistant.

For data analysis, the sensitivities and specificities of the methacholine challenge test for AHR were calculated for a range of  $PC_{20}$  cut points in 0.5 mg/mL increments from 1.0 mg/mL to 8.0 mg/mL, using physician diagnosis as the gold standard. Receiver operator characteristic (ROC) curves were constructed by plotting these values, and subsequently used to determine the best  $PC_{20}$  cut points conferring the greatest sensitivity plus specificity for AHR. The area under the ROC curve (AUC) was also measured as an indicator of the value of the methacholine challenge test as a diagnostic aid for asthma in this cohort. The AUC was determined using two methods, the trapezoidal rule and the Delong method. The trapezoidal rule derives the AUC directly from the ROC curve with the following formula:

$$\int_a^b f(x)dx \approx (b - a) \frac{f(a) + f(b)}{2}$$

The Delong method, as described by Hanley and Hajian-Tilaki<sup>21-22</sup>, estimates the AUC, along with its standard error and 95% confidence interval. AUC values from 0.90 to 1.00 indicate an excellent test, 0.80 to 0.90 describe a good test, 0.70 to 0.80 describe a fair test, and 0.60 to 0.70 describe a poor test. Values from 0.50 to 0.60 are considered a failure with the probability similar to that of a coin toss. The best  $PC_{20}$  cut point was determined by identifying the  $PC_{20}$  cut point that conferred the greatest sensitivity plus specificity out of all cut points examined. The data was analyzed as a whole, and stratified by sex and atopy. Atopic asthma was defined as a clinical diagnosis of asthma and a positive skin test, while non-atopic asthma was a diagnosis of asthma without a positive skin test. Data analysis for this project was done using Microsoft Excel and SAS version 9.2.

Ethics committees from the University of British Columbia and the University of Manitoba approved the 15-year assessments. Adolescents and parents provided written informed consent.

## **Results**

The baseline characteristics of the study population are summarized in Table 1. A total of 330 adolescents, 180 from the intervention group and 150 from the control group, returned to be assessed at a mean age of 14.7 years. Of these, 277 underwent the methacholine challenge test,

**STUDENT NAME:** *Maggie Ong*

while 325 did the skin prick test. In terms of their clinical status, 54 were diagnosed with asthma, 204 had atopy based on a positive skin test, 45 had atopic asthma, and 9 had non-atopic asthma. Table 1 includes the same demographic information of the cohort at 7 years of age. The sample size at 15 years of age however was smaller than that at 7 years of age, which comprised 380 children, despite efforts to persuade all former subjects to return.

The sensitivities and specificities of the methacholine challenge test according to physician diagnosis are listed in Table 2. PC<sub>20</sub>'s requiring greater concentrations of methacholine were associated with gains in sensitivity, but losses in specificity. For example, the concentration of 1.0 mg/mL conferred the lowest sensitivity of 22.0%, but the highest specificity of 98.7%. In contrast, the concentration of 8.0 mg/mL gave the highest sensitivity of 63.4%, but the lowest specificity of 75.8%. Analysis by sex and atopy produced similar results with the sensitivities increasing and the specificities decreasing progressively with higher concentrations of methacholine. The numbers and percentages of adolescents with PC<sub>20</sub>'s below each cut point defining AHR are also shown in Table 2.

ROC curves were constructed by plotting the sensitivity against 1-specificity for each PC<sub>20</sub> cut point examined in our study, as demonstrated in Figure 2. Separate ROC curves were created for asthma overall and asthma as stratified by sex and atopy. The AUC's for each ROC curve were calculated and are listed in Table 3. Since identical values were calculated using the trapezoidal rule and the Delong method, we report only the latter. The AUC for asthma overall was 0.73 (95% CI 0.65-0.82) which indicates that the methacholine challenge is a fair test. However, this observation is primarily driven by the AUC for atopic asthma which was 0.76 (95% CI 0.66-0.85). Unfortunately, a lower AUC of 0.57 (95% CI 0.36-0.78) was measured for non-atopic asthma, which describes it as a failure. Particularly amongst females with non-atopic asthma where the AUC was 0.50 (95% CI 0.27-0.72), the test has a probability only as good as a coin toss. On the contrary, a higher AUC of 0.71 (95% CI 0.15-1.28) was obtained for males with non-atopic asthma, which indicates that the methacholine challenge is a fair test.

The best PC<sub>20</sub> cut points conferring the greatest sensitivity plus specificity for physician-diagnosed asthma in high-risk adolescents, with their respective sensitivities and specificities, are summarized in Table 4. For asthma overall, the PC<sub>20</sub> cut point defining AHR was  $\leq 5.5$  mg/mL (sensitivity of 61.0% and specificity of 85.2%). The same PC<sub>20</sub> cut point of  $\leq 5.5$  mg/mL (sensitivity of 67.6% and specificity of 82.7%) was also measured for atopic asthma. However, for non-atopic asthma, a lower PC<sub>20</sub> cut point of  $\leq 3.0$  mg/mL was obtained for all adolescents (sensitivity of 28.6% and 94.9%) and females alone (sensitivity of 20.0% and specificity of 90.9%), while an even lower PC<sub>20</sub> cut point of  $\leq 1.5$  mg/mL (sensitivity of 50.0% and specificity of 100.0%) was determined for males alone. Of importance to note, the sensitivities of the test for non-atopic asthma were all less than or equal to 50%, which makes the methacholine challenge a very insensitive test for non-atopic asthma at 15 years of age.

The same outcomes were previously measured in cohort at 7 years of age. Comparison with results from 15 years of age shows that the sensitivities of the methacholine challenge test were higher, but the specificities were lower at 7 years of age, as shown in Table 4. For example, at 7 years of age, the sensitivity of the test for asthma overall was 80.0%, while the specificity was 47.9%. In contrast, at 15 years of age, the test had a sensitivity of 61.0% and specificity of

**STUDENT NAME:** *Maggie Ong*

85.2%. When the data was stratified by sex and atopy, a similar pattern of values was obtained, in which the sensitivity was higher, but specificity was lower at 7 years of age. Another observation is that at 7 years of age, the specificities of the test for non-atopic asthma were all below 50%, which makes the methacholine challenge a very non-specific test for non-atopic asthma.

The utility of the methacholine challenge test as an aid to diagnosing asthma was lower at 7 years of age compared to 15 years of age, as shown in Table 3. The AUC for asthma overall was 0.69 (95% CI 0.62-0.76), which indicates that it is a poor test. Likewise, when stratified by sex, the AUC for females with asthma overall was 0.70 (95% CI 0.60-0.80), again making it a poor test. Stratification by atopy produced an AUC of 0.70 (95% CI 0.61-0.79) for atopic asthma, and an AUC of 0.64 (95% CI 0.52-0.76) for non-atopic asthma, both indicating that it is a poor test.

Differences were also observed when comparing the best PC<sub>20</sub> cut points to assist clinicians in the diagnosis of asthma as the values measured at 7 years of age were lower compared to those obtained at 15 years of age, as demonstrated in Table 4. The best PC<sub>20</sub> cut point defining AHR in high-risk 7-year-olds was  $\leq 3.0$  mg/mL for all children (sensitivity of 80.0% and specificity of 47.9%) and females (sensitivity of 82.6% and specificity of 47.8%) with asthma. Likewise, a cut point of  $\leq 2.0$  mg/mL (sensitivity of 66.7% and specificity of 64.4%) was determined for males with asthma. These findings show that the best PC<sub>20</sub> cut point increases over time as children enter into adolescence.

## **Discussion**

The results of our study show that the utility of the methacholine challenge test as a diagnostic aid for asthma in this cohort improved from 7 to 15 years of age as predicted. This was confirmed by an increase in the AUC, and the sum of sensitivity and specificity of the test. The utility of the test however was unchanged in females over time as the AUC stayed relatively the same. On the contrary, there was a loss in value of the test in adolescents with non-atopic asthma, in particular females, as demonstrated by a decrease in AUC over time. Another finding was that the sensitivity of the test decreased, while the specificity increased from 7 to 15 years of age. Furthermore, the best PC<sub>20</sub> cut point defining AHR in high-risk adolescents at 15 years of age was  $\leq 5.5$  mg/mL, which is an increase from the cut point of  $\leq 3.0$  mg/mL measured at 7 years of age.

The improved utility of the methacholine challenge test from 7 to 15 years of age confirms that our hypothesis was correct. The value of the test appears to increase with age as children enter into adolescence. Similar results were derived from SAGE, as the utility of the methacholine challenge test improved from the ages of 7-10 to 11-14. The AUC in 7-10 year-olds of this cohort was 0.70<sup>11</sup>, while a higher AUC of 0.79 was measured in 11-14 year-olds<sup>12</sup>. Likewise, the sensitivity and specificity of the methacholine challenge test improved over time with increasing age. In 7-10 year-olds from this cohort, the sensitivity and specificity of the test were calculated to be 65.4% and 64.6% respectively<sup>11</sup>, whereas in 11-14 year-olds, the same respective values were 71.3% and 79.3%<sup>12</sup>.

**STUDENT NAME:** *Maggie Ong*

Age appears to be a major factor influencing the improved value of the methacholine challenge test in adolescents with asthma. A potential reason for this is the higher prevalence of AHR in asthmatic adolescents compared to asthmatic children. While AHR is present in 100% of adults with asthma<sup>10</sup>, it is not an essential or sufficient finding in children with asthma<sup>23</sup>. Thus, as asthmatic children enter adolescence and approach adulthood, a greater proportion of them develop AHR over time, resulting in a higher sensitivity and specificity of the test. Another factor influencing the increase in utility of the methacholine challenge test over time is the ease and accuracy of the clinical diagnosis of asthma in adolescence compared to childhood<sup>6</sup>. Adolescents diagnosed with asthma at 15 years of age in our study were more likely to have a correct diagnosis. In contrast, the overdiagnosis of asthma in childhood could have resulted in a lower sensitivity and specificity of the test at 7 years of age.

Another finding in our study was the increase in best PC<sub>20</sub> cut point defining AHR from 7 to 15 years of age, which is also consistent with our predictions. This result suggests that the best PC<sub>20</sub> cut point tends to increase over time as children approach adulthood. On the contrary, in SAGE, the best PC<sub>20</sub> cut point remained unchanged at  $\leq 4.0$  mg/mL from 7-10 years of age to 11-14 years of age<sup>11-12</sup>. This conflict between the two studies makes it difficult to make sense of any potential existing association between age and PC<sub>20</sub> cut points. It could result from the fact that our cohort is high-risk and that somehow the presence of a family history of asthma could have accelerated the increase in best PC<sub>20</sub> cut points with age. Despite the conflict in findings, to explain what was observed in our study, it seems biologically plausible that the best PC<sub>20</sub> cut point defining AHR would increase over time. It is also plausible that the increase in cut point happens close to the ages of 14-15 and the children in SAGE were on average slightly younger. The best PC<sub>20</sub> cut point defining AHR in adults is 8.0 mg/mL<sup>10</sup>, so children, who tend to have lower PC<sub>20</sub> cut points, would have cut points that progressively approach this value as they enter into adolescence and subsequently adulthood.

The results of our study were somewhat different when stratified by the sex. While the utility of the methacholine challenge test improved in males with asthma, there was no improvement in females alone. The AUC's remained relatively the same for females with asthma from 7 to 15 years of age. The contrary was observed in SAGE as there was a gain in the utility of test for females from 7-10 to 11-14 years of age, with an increase in AUC from 0.70 to 0.75<sup>11-12</sup>. Similar to our study however there was an increase in AUC for males in SAGE from 0.68 to 0.81 between the ages of 7-10 and 11-14 years<sup>11-12</sup>. The discrepancy between both studies once again raises the possibility that the high-risk nature of our cohort could have influenced our results. There was no difference observed between males and females with respect to the best PC<sub>20</sub> cut points defining AHR in adolescence, as a value  $\leq 5.5$  mg/mL was obtained for both sexes, which is an increase from 7 years of age.

The lack of improvement of the test for females in our study indicates that the test is more useful in adolescent males than adolescent females with asthma. This finding could be due to hormonal shifts and sex-specific differences in environmental exposures in adolescence that altered the pathophysiology of the disease and thus airway sensitivity to methacholine. The relationship between sex and AHR in adolescence has been studied. We know that AHR diverges between males and females at 11 years of age, with males recording progressively higher PC<sub>20</sub>'s than females, and plateaus at 13 years of age<sup>24</sup>. In addition, although the level of AHR declines in



**STUDENT NAME:** *Maggie Ong*

both sexes in adolescence compared to childhood, females retain the advantage of lower airway tone<sup>25</sup>. AHR is also more severe in post-pubertal females with asthma than in males<sup>24</sup>. The lack of improvement of the test in females in our study could also be due to methodology. Females show less airway responsiveness than males in inhalational challenges<sup>26</sup>, despite having smaller airways, and dilate their airways while taking a deep breath<sup>27</sup>. Existing knowledge however does not fully explain the sex difference observed in our study, so more research needs to be done to further understand the association between sex and AHR in adolescence.

In addition to sex, stratifying the data in our study by atopic status also produced different findings. Although the utility of the test improved for atopic asthma, the opposite was true for non-atopic asthma, particularly in females, as it decreased from 7 to 15 years of age. For males with non-atopic asthma however the utility of the test increased. Similar results were observed in SAGE as the usefulness of the test for atopic asthma improved from 7-10 to 11-14 years of age, whereas it decreased for non-atopic asthma, especially amongst females, over the same period of time. The AUC for atopic asthma increased from 0.74 to 0.86, while it decreased for non-atopic asthma overall from 0.62 to 0.60, and females with non-atopic asthma from 0.68 to 0.50<sup>11-12</sup>. Thus, results from our study and SAGE show that the methacholine challenge test is particularly useful in assisting the diagnosis of atopic asthma in adolescents, while the contrary is true for non-atopic asthma, particularly amongst females, in which the test has a probability equivalent to a coin toss.

The improved utility of the test for atopic asthma is reasonable as atopy and AHR are known to be strong risk factors for asthma. Atopy is present in most children and 50% to 70% of adult asthmatics<sup>28</sup>, while AHR is found in 100% of adult asthmatics<sup>10</sup>. The association between atopy, AHR, and asthma has been well studied. Atopy and AHR are strong predictors of the most severe wheezing phenotypes in childhood, which include intermediate wheeze and persistent wheeze<sup>29</sup>. Similarly, they are predisposing risk factors for adolescent-onset asthma<sup>30</sup>, in addition to asthma in adulthood<sup>31</sup>. Thus, children and adolescents who have been identified with atopy and AHR are likely to develop more severe asthma if these factors are present. The findings of our study also suggest that the association between atopy and AHR becomes progressively stronger as children enter into adolescence, which could imply that AHR is increasingly prevalent in those with atopic asthma over time. Unfortunately, there is no reliable source reporting prevalence of AHR in a population of adolescents in comparison to children with atopic asthma. Furthermore, this outcome cannot be measured in our cohort because of the bias created by the high-risk nature of the adolescents.

Contrary to what was observed for atopic asthma, the decrease in utility of the test for non-atopic asthma suggests that it should not be used as an aid in the diagnosis of asthma in adolescents with non-atopic asthma, especially females. At the present, there is no logical explanation for this finding, but it is possible to speculate. Perhaps differences in hormones and environmental exposures occurring in adolescence, as mentioned already, could have influenced the sex disparity in test utility for those with non-atopic asthma. Another possible reason is that AHR may not be as prevalent in adolescents compared to children with non-atopic asthma. Once again, there is no source reporting AHR prevalence in adolescents with non-atopic asthma as compared to children, and this cannot be measured in our cohort because it is high-risk. As we

**STUDENT NAME:** *Maggie Ong*

can see, more research needs to be done to elucidate the specific differences between males and females with non-atopic asthma.

Stratifying the data by sex and atopy produced lower best PC<sub>20</sub> cut points for non-atopic asthma in particular. At 15 years of age, the best PC<sub>20</sub> cut point for non-atopic asthma in adolescents overall and females alone was  $\leq 3.0$  mg/mL, while the cut point in males alone was  $\leq 1.5$  mg/mL. These values are lower than the cut point of  $\leq 5.5$  mg/mL for asthma overall. At 7 years of age, the cut points remained relatively the same after stratification. Contrary to our study, in SAGE, stratifying the data by sex and atopy gave best PC<sub>20</sub> cut points for non-atopic asthma that were higher than those for asthma overall and atopic asthma<sup>11-12</sup>. Furthermore, in SAGE, no increase was observed in the best PC<sub>20</sub> cut points from 7-10 to 11-14 years of age<sup>11-12</sup>. The conflict between both studies again suggests that the high-risk nature of our cohort could have influenced these results. However, of importance in our study, the sensitivities of the methacholine challenge test for non-atopic asthma at 15 years of age were all below 50%, which means that the test is insensitive and should not be used as a diagnostic aid in the first place.

The main recommendations derived from this study are that the methacholine challenge is fair test in assisting the diagnosis of asthma in high-risk adolescents at 15 years of age, and that its utility seems to improve over time from childhood to adolescence. The test is useful for atopic asthma, but not at all for non-atopic asthma, particularly amongst females, in which it is a failure with its value being equivalent to that of a coin toss. The discrepancy in utility of the test between atopic asthma and non-atopic asthma suggests that skin prick testing to assess for the presence of atopy might take precedence over the methacholine challenge test when it comes to deciding the order of clinical tests to perform in assisting the clinical diagnosis of asthma. Seeing that the test is useless in adolescent females with non-atopic asthma, there is no point in administering it to adolescent females who fail to show a positive skin prick test. Another recommendation obtained from this study is that the best PC<sub>20</sub> cut point defining AHR in high-risk adolescents with asthma at 15 years of age is  $\leq 5.5$  mg/mL, and that this cut point appears to increase over time from childhood to adolescence. Despite being close to adulthood, the best PC<sub>20</sub> cut point for high-risk adolescents at 15 years of age as determined in our study is far from the cut point of  $\leq 8.0$  mg/mL that is recommended for adults.

Limitations to our study include the high-risk nature of our cohort, a small sample size, potential confounding variables, and human error in data collection. The high-risk nature of our cohort does not permit the application of our results to the general population. It also does not allow us to reliably verify our results with similar analyses done in other cohorts that are not exclusively high-risk, such as SAGE. When it comes to sample size, the population we examined was somewhat small as it consisted of 330 adolescents who returned to be assessed. However, at the initiation of the study, the population was much larger as it consisted of 545 families, but many were unfortunately lost to follow-up over the years. A larger sample size would have increased the accuracy of our results. Another limitation is potential confounding variables that were not included in our analysis. Sex and atopy were the only two variables that our data was stratified according to as they were the most important variables affecting the outcomes. Nonetheless, there were other variables that could have influenced our results, such as ethnicity, maternal or paternal history of asthma, city of residence, or even group assignment. Human error is another

**STUDENT NAME:** *Maggie Ong*

possible limitation in our study which could have decreased the accuracy of data we obtained from the physician assessment, methacholine challenge test, and skin prick test.

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**STUDENT NAME:** *Maggie Ong*

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**Table 1: Baseline characteristics of the CAPPS cohort at 7 and 15 years of age.**

<b>7 years (Wave 3)</b>		<b>15 years (Wave 4)</b>	
Returned for follow-up	N = 380	Returned for follow-up	N = 330
Males	177 (46.6)	Males	183 (55.5)
Females	203 (53.4)	Females	147 (44.5)
Mean age (years)*	7.0	Mean age (years)	14.7
Ethnic origin		Ethnic origin	
Whites	301 (79.2)	Whites	269 (81.5)
Non-Whites	79 (20.8)	Non-Whites	61 (18.5)
Family history of asthma*	N = 292	Family history of asthma*	N = 216
Mother with asthma	162 (55.5)	Mother with asthma	147 (68.1)
Father with asthma	130 (44.5)	Father with asthma	69 (31.9)
Site		Site	
Winnipeg	194 (51.1)	Winnipeg	173 (52.4)
Vancouver	186 (48.9)	Vancouver	157 (47.6)
Assessments		Assessments	
Methacholine challenged	350 (92.1)	Methacholine challenged	277 (83.9)
Skin tested	367 (96.6)	Skin tested	325 (98.5)
Clinical Status		Clinical Status	
Asthma	71 (18.7)	Asthma	54 (16.4)
Atopy	159 (41.8)	Atopy	204 (61.8)
Atopic asthma	51 (13.4)	Atopic asthma	45 (13.6)
Non-atopic asthma	20 (5.3)	Non-atopic asthma	9 (2.7)
Group assignment		Group assignment	
Intervention	202 (53.2)	Intervention	180 (54.5)
Control	178 (46.8)	Control	150 (45.5)

\* Data on family history of asthma was not available for many cases

STUDENT NAME: *Maggie Ong*

**Table 2: Sensitivity and specificity of AHR according to physician-diagnosed asthma at ages 15 using 0.5 cut points for PC20.**

Cut points PC20 (mg/ml)	No asthma N = 236	Asthma N = 41	Sensitivity	Specificity
8.0	194 (82.2)	83 (202.4)	63.4	75.8
7.5	198 (83.9)	79 (192.7)	61.0	77.1
7.0	200 (84.7)	77 (187.8)	61.0	78.0
6.5	205 (86.9)	72 (175.6)	61.0	80.1
6.0	210 (89.0)	67 (163.4)	61.0	82.2
5.5	217 (91.9)	60 (146.3)	61.0	85.2
5.0	225 (95.3)	52 (126.8)	48.8	86.4
4.5	230 (97.5)	47 (114.6)	46.3	88.1
4.0	232 (98.3)	45 (109.8)	43.9	88.6
3.5	234 (99.2)	43 (104.9)	43.9	89.4
3.0	243 (103.0)	34 (82.9)	39.0	92.4
2.5	245 (103.8)	32 (78.0)	39.0	93.2
2.0	254 (107.6)	23 (56.1)	34.1	96.2
1.5	259 (109.7)	18 (43.9)	31.7	97.9
1.0	265 (112.3)	12 (29.3)	22.0	98.7

**Table 3: Areas under the curve by group and age at follow-up.**

Group	Age 7		Age 15	
	ROC AUC	95% CI	ROC AUC	95% CI
Total asthma	0.69	0.62-0.76	0.73	0.65-0.82
Atopic asthma	0.70	0.61-0.79	0.76	0.66-0.85
Non-atopic asthma	0.64	0.52-0.76	0.57	0.36-0.78
Total asthmatic males	0.68	0.58-0.77	0.77	0.65-0.89
Atopic asthmatic males	0.68	0.57-0.79	0.76	0.63-0.89
Non-atopic asthmatic males	0.61	0.43-0.78	0.71	0.15-1.28
Total asthmatic females	0.70	0.60-0.80	0.69	0.56-0.82
Atopic asthmatic females	0.71	0.56-0.86	0.75	0.60-0.89
Non-atopic asthmatic females	0.68	0.52-0.85	0.50	0.27-0.72

**Table 4: Methacholine challenge test best PC<sub>20</sub> cut points by group and age at follow-up.**

Group	Age 7		Age 15	
	Best cut point	Sensitivity/Specificity	Best cut point	Sensitivity/Specificity
Total asthma	≤ 3.0 mg/mL	80.0%/47.9%	≤ 5.5 mg/mL	61.0%/85.2%
Atopic asthma	≤ 2.5 mg/mL	78.3%/53.5%	≤ 5.5 mg/mL	67.6%/82.7%
Non-atopic asthma	≤ 3.0 mg/mL	78.9%/48.6%	≤ 3.0 mg/mL	28.6%/94.9%
Total asthmatic males	≤ 2.0 mg/mL	66.7%/64.4%	≤ 5.5 mg/mL	61.9%/87.6%
Atopic asthmatic males	≤ 2.0 mg/mL	71.0%/59.0%	≤ 5.5 mg/mL	63.2%/84.5%
Non-atopic asthmatic males	≤ 2.0 mg/mL	54.5%/68.2%	≤ 1.5 mg/mL	50.0%/100.0%
Total asthmatic females	≤ 3.0 mg/mL	82.6%/47.8%	≤ 5.5 mg/mL	60.0%/81.8%
Atopic asthmatic females	≤ 2.5 mg/mL	73.3%/60.0%	≤ 5.5 mg/mL	73.3%/80.0%
Non-atopic asthmatic females	≤ 3.0 mg/mL	87.5%/49.0%	≤ 3.0 mg/mL	20.0%/90.9%

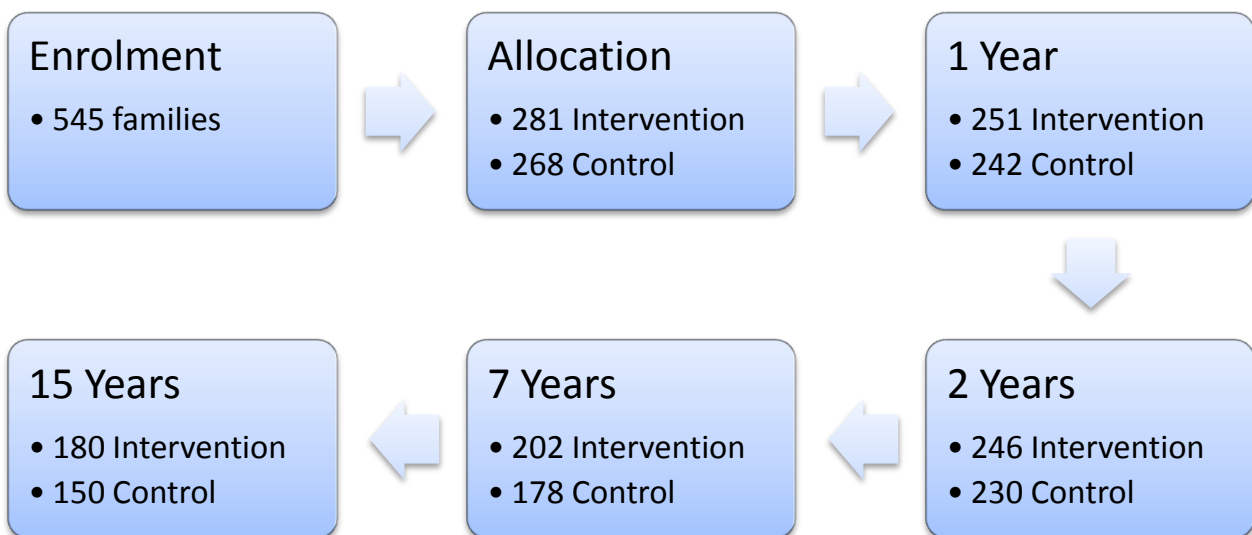


Figure 1: Retention numbers at each wave of CAPPs since its initiation. A total of 545 families were initially enrolled, with 281 infants randomized to the intervention group and 268 to the control group. Assessments were done at 1, 2, 7, and 15 years of age. Many were lost to follow-up gradually over the years. At 15 years of age, 330 adolescents returned to be assessed, with 180 from the intervention group and 150 from the control group.

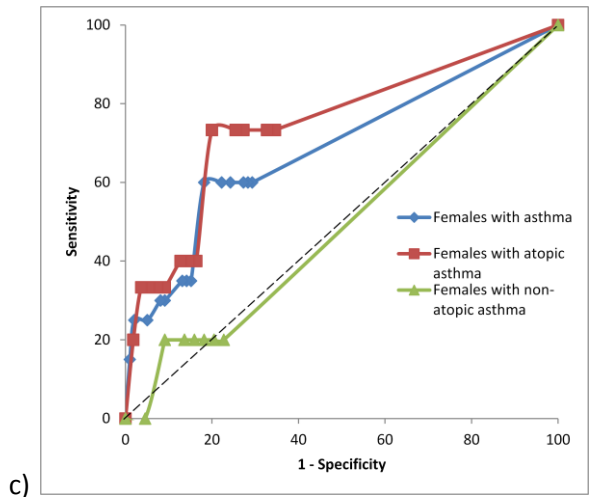
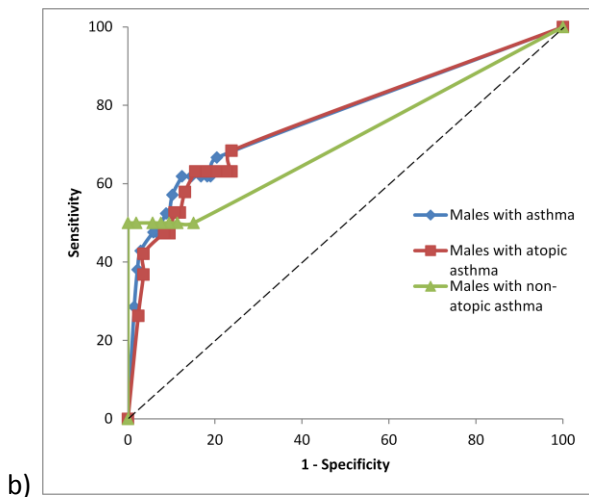
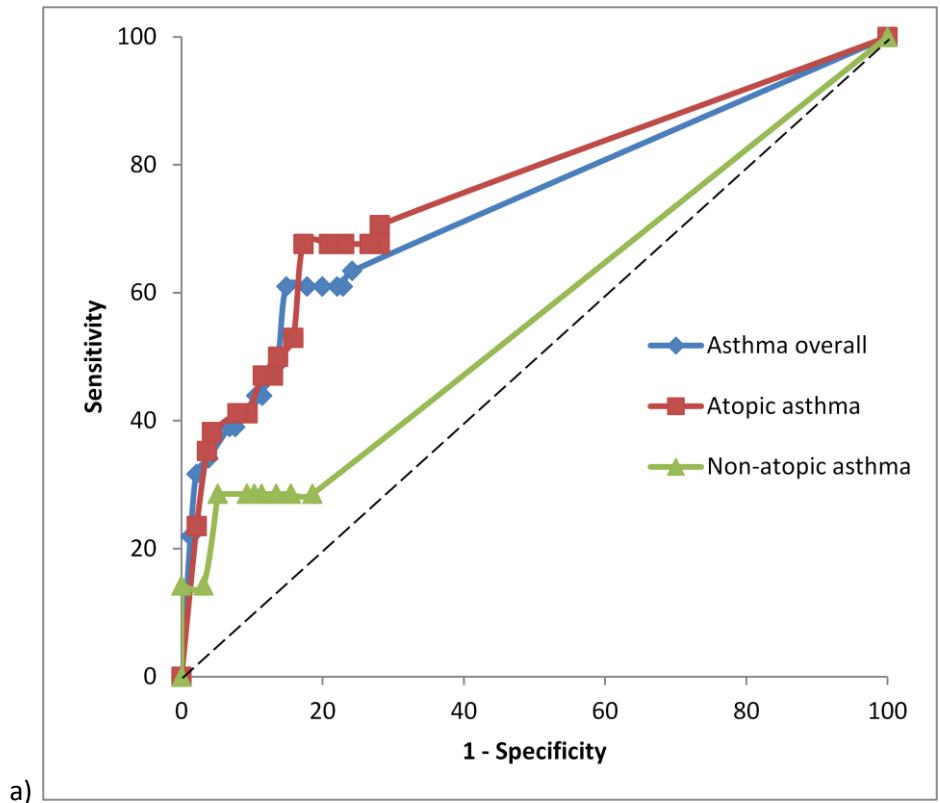


Figure 2: a) ROC curves for asthma overall (blue diamonds; AUC = 0.73), atopic asthma (red squares; AUC = 0.76), and non-atopic asthma (green triangles; AUC = 0.57) at 15 years of age. b) ROC curves for males with asthma (blue diamonds; AUC = 0.77), atopic asthma (red squares; AUC = 0.76), and non-atopic asthma (green triangles; AUC = 0.71) at 15 years of age. c) ROC curves for females with asthma (blue diamonds; AUC = 0.69), atopic asthma (red squares; AUC = 0.75), and non-atopic asthma (green triangles; AUC = 0.50) at 15 years of age.