Etiology of Viral Wheeze Determines Risk for Childhood Asthma

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Disclosure of relevant commercial interests: None

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Phenotypes of childhood wheezing

- Transient early wheeze
- Non-allergic asthma or recurrent wheezing
- Allergic asthma

Questions

1. What are the developments in viral diagnostics of acute wheezing?

2. Why are viral risk markers for asthma needed?

3. Is there a link between viral etiology of wheezing and development of asthma?

4. What does positive HRV PCR mean?

5. Which factors are linked to HRV induced wheezing?
1. What are the developments in viral diagnostics of acute wheezing?
Discovery of respiratory viruses: new wave during 21st century due to molecular methods

- Influenza virus 1933
- Coxsackie virus 1948
- Echovirus 1951
- Adenovirus 1953
- RSV 1956
- Rhinovirus 1956
- Parainfluenza virus 1956
- Coronavirus 1965
- Torque Teno virus 1997
- Metapneumovirus 2001
- Coronavirus: SARS, NL63 & HKU1 2002-2005
- Bocavirus 2005
- Polyomaviruses: KI & WU 2007
- Rhinovirus C groups 2007-

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### The virus etiology of childhood wheezing over 5 decades

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Cu</th>
<th>Ag</th>
<th>Sero</th>
<th>PCR</th>
<th>RSV</th>
<th>HRV</th>
<th>EV</th>
<th>PIV</th>
<th>Flu</th>
<th>AdV</th>
<th>CV</th>
<th>MPV</th>
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<th>PyV</th>
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<td>7</td>
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<td>Nieminen et al. (2012), unpubl</td>
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</table>

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**Viral wheeze is really viral wheeze**
Early wheezing is a viral illness

RSV dominates in infants aged <12 months
HRV dominates in children aged ≥12 months

RSV, $P < 0.0001$ for trend

- 45/57 (0-5 mo) Episode number not known
- 18/45 (6-11 mo) Recurrent episode
- 21/98 (12-23 mo) First episode
- 12/53 (24-35 mo) First episode

HRV, $P = 0.035$ for trend

- 14/52 (0-5 mo) Episode number not known
- 15/45 (6-11 mo) Recurrent episode
- 39/98 (12-23 mo) First episode
- 24/53 (24-35 mo) First episode


Early wheezing is a viral illness

RSV dominates in infants aged <12 months
HRV dominates in children aged ≥12 months

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Bronchiolitis is particularly associated with HRV in predisposed children

• **Subjects:** Infants with parental asthma/allergy & recurrent resp. infections during 1st yr of life, n = 27

• **Results:**
  – Of wheezing infants, **78% wheezed at least once with HRV**, 70% with RSV and \(< 39\%\) with other viruses
  – **47% of wheezing episodes were associated with HRV**, 24% with RSV, and \(< 13\%\) with other viruses

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HRVs are highly diverse, HRV-C recently discovered

**HRV:** Subgroups A, B & C, 100 serotypes, >150 genotypes

**RSV:** Single serotype with 2 major antigenic subgroups A and B, >12 genotypes


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HRV-C has a high clinical impact

- HRV-C has constituted almost half of all HRV-associated hospitalizations Miller et al. JACI 2009, Peltola et al. JMV 2009


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Summary: What are the developments in viral diagnostics of acute wheezing?

- A renaissance of virus discovery; 100% detection rate reached
- The prevalence of HRV is 20-40% in bronchiolitis (ER/hospital)
- Up to 50-80% in bronchiolitis if predisposed
2. **Why** are viral risk markers for asthma needed?
Hospitalization for bronchiolitis has been linked to asthma up to age 27 years.

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Piippo-Savolainen & Korppi Acta Paediatr 2007,
Ruotsalainen et al. Allergy 2010
Early wheezing is an independent risk factor of adulthood asthma

The risk is further increased if a child has:
• Parental asthma
• Atopy related factors: aeroallergen sensitization, atopic dermatitis, eosinophilia
• Recurrent wheezing at age <2 years
• Decreased lung function
• Human rhinovirus (HRV) induced wheezing

Any sensitization (left) and aeroallergen sensitization (right) develop slowly.
3. Is there a link between viral etiology of wheezing and development of asthma?
Long-term follow-up studies on HRV and RSV induced wheeze

RSV vs non-RSV
- Stein Lancet 1999
- Piippo-Savolainen Pediatr Int 2007
- Valkonen Allergy 2009

HRV vs RSV / non-HRV
- Kotaniemi-Syrjänen JACI 2003
  - Hyvärinen PPul 2005
- Lemanske JACI 2005
  - Jackson JACI 2008
- Lehtinen JACI 2007
  - Lukkarinen unpubl.
- Kusel JACI 2007
  - Kusel ERJ 2012
- Midulla ERJ 2011
3.1. **RSV vs non-RSV bronchiolitis:**
link to asthma?
### LRTI <3 years and infrequent (top) and frequent (bottom) wheeze

Tucson, Arizona

<table>
<thead>
<tr>
<th></th>
<th>6 yr</th>
<th>8 yr</th>
<th>11 yr</th>
<th>13 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RSV</strong></td>
<td>3.2 *</td>
<td>2.5 *</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Neg test</strong></td>
<td>1.5</td>
<td>1.3</td>
<td>1.6</td>
<td>1.9†</td>
</tr>
</tbody>
</table>

### OR adjusted for sex, maternal education, family history of asthma, allergy skin tests at yr 6, birthweight, and current maternal smoking

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Stein et al. Lancet 1999
Adult asthma after non-RSV vs RSV induced bronchiolitis: 20-year prospective follow-up
Kuopio, Finland

- N = 54, hospitalized for bronchiolitis at age < 2 yrs
- Re-studied at median age 19 years
- Outcome: adult asthma; 2 definitions
- Asthma: non-RSV 41-50% vs RSV 18-27%
- In adjusted analysis, non-RSV etiology increased asthma risk by both strict (OR 8.3) and less strict (OR 7.9) criteria

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Lung function and BHR 11 years after hospitalization for bronchiolitis

Kuopio, Finland

- Forced vital capacity:
  - RSV 94% vs non-RSV 100%

- Bronchial hyperresponsiveness:
  - early sensitization (OR 12.6)
  - maternal smoking during pregnancy (OR 4.6)
  - early atopic dermatitis (3.5)
Recurrent wheezing requiring long-term asthma medication during first 1, 2, and 3 years after the hospitalization for RSV vs non-RSV bronchiolitis

Turku, Finland

RSV: n = 75, non-RSV: n = 69

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Valkonen et al. Allergy 2010
3.2. **HRV vs RSV / non-HRV bronchiolitis / wheeze: link to asthma?**

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The first report on the link between HRV bronchiolitis and asthma
Kuopio, Finland

- Population based, hospitalized, \( N = 66 \)
- Age \(<2\) yrs at recruitment, \(5-8\) yrs at follow-up visit
- \(27\) \((41\%)\) had asthma at school-age
- **OR 4.1 for school-age asthma in HRV group**
  (compared to non-HRV, adjusted for age, sex, and atopic dermatitis)
- No longer significant at 11-yr follow-up (Hyvärinen et al. Pediatr Pulmonol 2005)
HRV wheezing during the 1st 3 yrs of life is highly associated with asthma at age 6 yrs

In the 1st year of life, HRV wheezing had OR 10 for wheezing at age 3 years

In the 3rd year of life, HRV wheezing had OR 25.6 for asthma at age 6 years

Lemanske et al. J Allergy Clin Immunol 2005
Jackson et al. Am J Respir Crit Care Med 2008
Jartti BLF 2012
Early atopic sensitization is linked to current wheeze at 5 years of age
Perth, Australia

<table>
<thead>
<tr>
<th>Any wheezy LRI with</th>
<th>Never atopic</th>
<th>Atopic by age of 2 yrs</th>
<th>Atopic after age 2 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRV or RSV</td>
<td>0.8</td>
<td>4.1</td>
<td>0.9</td>
</tr>
<tr>
<td>HRV</td>
<td>1.6</td>
<td>3.2</td>
<td>2.1</td>
</tr>
<tr>
<td>RSV</td>
<td>1.6</td>
<td>3.6</td>
<td>N ↓</td>
</tr>
</tbody>
</table>

Odds ratios, n = 198

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Early atopic sensitization is linked to asthma at 10 years of age

Perth, Australia

<table>
<thead>
<tr>
<th>Whole population</th>
<th>Atopic by age of 2 yrs</th>
<th>Atopic after 2 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Febrile infection</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>LRI with fever</td>
<td>4.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Wheezy / febrile LRI</td>
<td>3.0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Odds ratios, n = 147

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Kusel et al. Eur Respir J 2012
1-year outcome of the 1st wheezy episode at age <3 years

Turku, Finland

Jartti BLF 2012  Bold line = prednisolon; thin line = placebo  Lehtinen et al. JACI 2007
7-year outcome of the 1st wheezy episode at age <3 years
Turku, Finland

Jartti BLF 2012

Follow-up time (months)

Probability of asthma

Bold line = prednisolone thin line = placebo

Lukkarinen et al. unpublished
7 year outcome of the 1st wheezy episode at age <3 yrs
Turku, Finland

• Independent risk factors for asthma:
  – **HRV**: HR 4.5 (compared to RSV)
  – other than RSV or HRV etiology: HR 3.3
  – any sensitization: HR 3.3
  – age <1 year: HR 2.3

• Prednisolone recipients had 54% lower probability of asthma in HRV group *post hoc*
One year outcome of bronchiolitis (1st episode) at age <12 months

Rome, Italy

• Outcome = recurrent wheezing, i.e. >2 physician-verified episodes of wheezing during a one year follow-up

• n = 313, all hospitalized

• Results: risk factors for recurrent wheezing
  – B-eos count >0.4 x 10^9/L, OR 4.9
  – HRV, OR 4.0
  – Family history for asthma, OR 2.7

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Summary: Is there a link between viral etiology of wheezing and development of asthma?

- Any virus: 5 fold risk (~30%)
- Non-RSV vs RSV: 0 - 4.5 fold risk
- HRV vs RSV / non-HRV: 3 - 10 (-25) fold risk
4. What does positive HRV PCR mean?
The interpretation of positive PCR results has been confounded by multiple coexisting viruses in symptomatic subjects (up to 43%) and by high virus detection rates in asymptomatic subjects (up to 40–68%).

PCR has increased virus detection rates in asymptomatic subjects

- Literature search from 1965 to present
- 51 articles, n ~ 15,000 for samples
- The prevalences of viruses in respiratory samples of asymptomatic subjects:

<table>
<thead>
<tr>
<th>Virus</th>
<th>PCR +</th>
<th>Conventional +</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>HRV</td>
<td>15% (365 / 2416)</td>
<td>1.5% (255 / 14669)</td>
<td>0.0001</td>
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<tr>
<td>RSV</td>
<td>2.6% (51 / 1974)</td>
<td>0.7% (23 / 3175)</td>
<td>0.0001</td>
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<tr>
<td>AdV</td>
<td>5.3% (103 / 1958)</td>
<td>1.8% (40 / 2175)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

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Do same HRVs persist or re-occur?

Viral findings in 27 infants with frequent (>5) moderate-to-severe respiratory illnesses during the 1st year of life

Jartti et al. 
Eur Respir J 2008 
Jartti BLF 2010
Jartti et al. Eur Respir J 2008:
Same HRV strain (3%, 4/123) or any virus (5%, 13/244) rarely reoccurred in infants with recurrent illnesses; only 4 double HRV infections were found

Kaiser et al. AJRCCM 2006:
3/68 (4%) lung transplant recipients with graft dysfunctions had persistent HRV infection in upper and lower respiratory specimens over a 12-month period
Picornavirus longitudinal surveillance

- ≥3 seasons, n = 15
- 192/740 (26%) weekly samples were picornavirus positive
- 70% of positives were symptomatic and 30% asymptomatic
- 39% of infections occurred with "runs" of positives
HRV RNA may take 5-6 weeks and enterovirus RNA 2-3 weeks to disappear from nasal mucus in wheezing children.

No association to symptoms or use of systemic corticosteroid.

n = 84
HRV, mixed and all PCR+ viral findings correlate with the severity of illness


Viral findings in the 236 study samples of 27 frequently ill infants.

Similar finding by Mansbach Arch Pediatr Adolesc Med 2012: HRV alone was linked to prolonged LOS for bronchiolitis compared RSV alone.

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HRV-PCR+ correlates with systemic cytokine responses in young wheezing children

• **PATIENTS:** age of 3-35 months, 1st or 2nd wheezing episode. Asymptomatic controls (n=11) for HRV-group.

• **METHODS:**
  – Th1-type (IFN -gamma, IL-2, IL-12),
  – Th2-type (IL-4, IL-5, IL-13) and
  – Treg-type (IL-10) cytokines were studied from acute and convalescence phase serum samples of sole HRV (n=23) and sole RSV affected hospitalized wheezing children (n=27).

• **RESULTS:** HRV-group had markedly higher IFN-gamma levels than RSV-group or the controls.

Jartti BLF 2012

Jartti et al. Respir Res 2009
Serum IL-10 peaks in young children with acute wheezing

Jartti et al. Respir Res 2009

<table>
<thead>
<tr>
<th>Sole Infection</th>
<th>n</th>
<th>Acute (pg/mL)</th>
<th>2 weeks (pg/mL)</th>
<th>Difference</th>
<th>P</th>
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<tbody>
<tr>
<td>HRV</td>
<td>9</td>
<td>75</td>
<td>32</td>
<td>-44</td>
<td>0.039</td>
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<tr>
<td>RSV</td>
<td>10</td>
<td>54</td>
<td>9.1</td>
<td>-50</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Medians

Similar finding by Grissell et al. AJRCCM 2005:
- IL-10 mRNA was increased in acute asthma and reduced on recovery

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4.1. Is HRV able to infect lower airways?
HRV is frequently found in the lower airways of young children with asthmalike symptoms, and it correlates with airway resistance

- **Patients:** age 3-26 mo, recurrent asthmalike symptoms
- **Methods:** Body plethysmography (n = 201), bronchoscopy (n = 68), bronchial biopsies (n = 59) for in situ HRV detection
- **Results:**
  - HRV+ in 45% of cases
  - Decreased airway conductance: 86% vs 58%, HRV + vs −, $P = 0.037$
  - Prior respiratory symptoms correlated with HRV+ cases, $P = 0.036$

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HRV is present in the lower airways of children with tracheostomies, without the possibility of nasal contamination

- **Tracheostomy children with cold symptoms:** HRV was detected by PCR from the nasal and tracheal secretions of 3/7 children

- **Asymptomatic controls:** HRV was not detected by PCR in any samples of nasal secretions, but was isolated from 4/16 samples of tracheal secretions.

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Simons et al. Pediatr Allergy Immunol 2005
Positive HRV-PCR probably means **symptomatic or asymptomatic infection** in children

- Correlates positively with severity of illness and immune responses
- Persistent, recurrent and double HRV infections are rare
- HRV is able to infect lower airways
5. Which factors are linked to HRV-bronchiolitis / wheeze?
Atopic characteristics are closed linked to HRV-wheeze in children aged 3-35 months

<table>
<thead>
<tr>
<th></th>
<th>Sole HRV (n=40)</th>
<th>Sole RSV (n=38)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>1.4</td>
<td>0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atopic</td>
<td>44%</td>
<td>8%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B-Eos, /mm(^3)</td>
<td>0.44</td>
<td>0.086</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FeNO, ppb</td>
<td>8.6</td>
<td>5.4</td>
<td>0.013</td>
</tr>
<tr>
<td>Atopic eczema</td>
<td>25%</td>
<td>6%</td>
<td>0.042</td>
</tr>
<tr>
<td>1st episode</td>
<td>70%</td>
<td>89%</td>
<td>0.033</td>
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</tbody>
</table>

Similar findings by: Korppi et al. PIDJ 2004: HRV linked to atopic eczema  
Rakes et al. AJRCCM 1999: HRV linked to Eos/ECP, but not to sensitization  
Kusel et al. JACI 2007, Jackson AJRCCM 2011: HRV linked to sensitization  
Carroll et al. JACI 2012: maternal atopic asthma  

Jartti BLF 2012  
Inverse relationship of cord blood PHA-induced IFN-gamma secretion to number of viral infections in the first year of life

Basal cells of differentiated bronchial epithelium are more susceptible to HRV infection

• The susceptibility of different cell types to HRV infection was analysed in vitro

• Compared with suprabasal cells, basal cells had:
  – more HRV+ cells (4-fold increase).
  – more HRV RNA per cell (2-fold increase).
  – higher expression of ICAM-1 (4-fold increase).

• There was greater replication of HRV in culture models of epithelial injury compared with intact cell layer.

Jartti BLF 2012
HRV is associated with asthma in hospitalized young children

592/812, 73% enrolled, population based, PCR diagnostics

Jartti BLF 2012
Miller et al. J Infect Dis 2007
Transcriptional profiles correlate with HRV and RSV etiology and disease severity in children bronchiolitis


Unsupervised Cluster Analysis

Predictor genes discriminate RSV vs HRV with 94% accuracy

Correlations: CRSS and MDTH

$\begin{align*}
r &= 0.4 \\
p &< 0.001
\end{align*}$
HRV-wheeze in young children is likely to be associated with
- atopy, B-eos, eczema, FeNO
- low IFN-γ
- recurrent wheeze / asthma
- epithelial damage *in vitro*
- response to corticosteroid *post hoc*
Finally

- HRV is commonly associated with early wheezing, especially in atopic individuals (20%-80%)
- Overall, bronchiolitis increases the risk of asthma, but the risk further increases by non-RSV etiology, and especially by HRV etiology
- HRV-wheeze is associated with many asthma-associated characteristics, e.g. atopic characteristics, low interferon-γ levels, and epithelial damage.

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Thank you