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**Title:** Phenotype consensus is required to enable large-scale genetic consortium studies of food allergy

**Text:**

To the Editor,

Recent genome-wide association studies (GWAS) have identified multiple candidate variants for food allergy (FA).<sup>1-3</sup> In February 2019, we convened to develop an International FA Consortium (InFAC) for genetic research (Vancouver, Canada). The main goal of InFAC was to enable large-scale international collaborations on FA genetics, since polygenic diseases require large samples to identify associated genetic variants. One of the barriers addressed was the need for consensus phenotype definitions, as direct comparisons of previous findings are complicated by heterogeneity in the FA definitions applied. In particular, asymptomatic sensitized individuals and self-reported FA without laboratory testing pose significant challenges. Another key issue is the definition of appropriate

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controls which range from bio-banked general population controls to non-atopic controls with negative sensitization testing or absence of clinical history.

The double-blind, placebo-controlled food challenge (DBPCFC) is the gold standard for FA diagnosis, but some large-scale genetic resources may not have these data and it may be difficult to find an adequate number of individuals meeting this stringent FA definition. Some investigators have performed placebo-controlled food challenges without double-blinding or utilized open oral food challenges (OFC), both of which still provide strong clinical support for a diagnosis of FA. Use of standard cut-offs for *in vivo* and *in vitro* diagnostic tests as a surrogate for DBPCFC is not well established, because positive predictive value (PPV) cut-offs vary greatly across populations. We perceive the definition of FA phenotype is a major barrier towards making progress in FA genetic research. A key outcome from this meeting was to develop consensus around FA inclusion criteria for large-scale genetic research.

Two key consensus statements emerged regarding large-scale genetic FA research and are presented in Table 1a.

Convincing history and specific IgE to define food allergy:

What determines a convincing history of an IgE-mediated allergic reaction to a specific food is controversial. In practice, allergists use experienced clinical judgement regarding history, symptoms, and testing to make a diagnosis of FA. Several definitions and scores describing an allergic response were considered, including the World Allergy Organization definition of anaphylaxis (online supplement)<sup>4</sup> and the Astier score for reaction severity<sup>5</sup> (Table S2), but neither included all criteria deemed to be essential for a convincing history of FA. We therefore agreed that for the purposes of phenotyping for genetic studies, a convincing history should involve: *i*) exposure by ingestion of the food allergen *ii*) onset of symptoms within 2 hours of exposure with resolution within 24 hour, and *iii*) only objective symptoms (Table 1b). Two organ system involvement is not required and treatment is not considered. Local symptoms through contact of the food allergen with the skin or oral mucosa are

not sufficient. Reactions solely identified by subjective symptoms (Table 1b) or linked to unclear exposure (i.e.: after consuming multiple food allergens and cross-contamination) (Table 1b) are not considered convincing.. Some subjective symptoms were identified by allergists in the group as having weight in a clinical history of FA, but most felt that these symptoms and anaphylaxis would be accompanied by objective symptoms.

Tier 4 of statement 2 is the most controversial as the challenge is to define “minimal criteria” for FA. Many large-scale genetic studies lack a detailed clinical history but have laboratory testing, allowing the allergic individuals in these studies to be captured by Tier 4. While we realize that this may lead to the inclusion of some individuals with asymptomatic sensitization, there was consensus that Tier 4, using the currently accepted 95% PPV cut-offs for SPT or sIgE (Table 1c, Table S1 online supplement), will generate a reasonable level of certainty to allow international collaboration at the most inclusive level. However, there is a trade-off as categories become less stringent – available samples will increase, but at the cost of potential misclassification.

Sample size requirements, food allergy phenotyping and sample type:

The mode of inheritance, penetrance of risk genes and their frequency in the study population determine sample size requirements. For GWAS, sample size requirements depend greatly upon the research question being asked, the effect size, and the comparator group. Risk variants with large effect sizes do not require as large of a sample size, but many polygenic diseases do not have risk variants with large effect sizes. The genetic model assumed in the experiment is key and could include: 1) genetic risk variants exist for development of FA to a specific food 2) genetic risk variants exist that are common to developing all food allergies, but the specific allergy is determined by non-genetic factors 3) genetic risk variants exist that are common to the development of all atopic diseases (atopic dermatitis, allergic asthma, allergic rhinitis, eosinophilic esophagitis) but which one develops is determined by interaction effects either genetic (gene-gene) or non-genetic (gene-environment) factors 4) a combination of all of these assumptions.

Control group phenotypes also play a key role in sample size requirements: hypercontrols – individuals who have no history of FA, or atopic diseases, and laboratory testing that indicates that they are not sensitized to foods – are helpful to increase power to detect genetic variants. However, it would be difficult to separate genetic risk factors for atopic tendency from those that cause FA, and there may be an age discrepancy and generational differences between cases. Population-based controls may also be used, with consideration of potential misclassification; prevalence of the FA being studied may be low in the population, but the combined effect of multiple atopic conditions, some of which may be self-resolving, may not be negligible. Use of controls with atopic diseases for investigation of FA may be difficult given the waxing and waning conditions of the atopic march.

Case and control recruitment can be costly (both monetarily and in time) due to the diagnostic history, testing and follow-up required to achieve well-defined allergic phenotypes. Smaller sample sizes may have the power to detect genetic variants of interest in circumstances where the genetic risk factor has a large effect size and there are well-phenotyped cases and hypercontrols, but sample size requirements will vary depending upon the genetic model, the population studied, and the question asked.

For genetic studies, samples can be taken from a variety of tissue types. However, for epigenetic studies, sample type is key as each tissue will exhibit a specific epigenetic signature. Some sample types, for example blood samples, may be more difficult to use in epigenetic studies due to the variety of cell populations within the sample. For FA, which presents with a diverse variety of effects in various systems and thus has no single tissue affected, most studies have focused on blood samples. For epigenetic studies the sample size is dependent on the purity of the tissue and how enriched that tissue is for the disease-associated signal, in addition to the variables mentioned previously for GWA studies.

Conclusion:

Recent publications on FA genetics have identified many potential loci for atopic diseases. However, the underlying genetic model for these diseases is unknown. Atopic conditions may share common genetic susceptibility, and environmental factors may determine which specific atopic disease develops; alternatively, each atopic disease may have its own risk variants, with transient and persistent forms differing in genetic susceptibility (Figure 1b).

These complex questions cannot be answered by studies with small samples, and genetic and molecular studies are inherently costly endeavors. Pooling data across studies is a cost-effective method to increase power. This will require a harmonization of phenotyping to ensure that efforts are not diluted by varying phenotypes. A balance between the gold standard and well-accepted global clinical and laboratory surrogate markers is required, as well as data on the transferability of IgE values from different platforms. While the gold standard of phenotyping of FA is DBPCFC, for research purposes we propose various levels of phenotyping to maximize potential collaborations. Findings can be subsequently verified in FA diagnosed by the gold standard.

Some may question the utility of genetic markers for FA due to ample evidence on the role of the environment in the development of allergies.<sup>6</sup> In an era of precision medicine, biologics, oral, epicutaneous and sublingual immunotherapy, genetic markers may significantly enhance decision-making.<sup>7</sup> Genetic susceptibility may have a role in the development of natural or treatment-induced tolerance.

Understanding the genetic architecture of FA remains a research imperative as accurate polygenic models may inform differing natural histories (i.e. resolution versus persistence of FA), biomarker discovery, and identify patients most likely to benefit from targeted primary prevention and immunotherapy. This aligns with efforts to provide more specific recommendations among patient subgroups, as considerable heterogeneity exists even among similar FA phenotypes. Thus, it is of utmost importance to pursue joint global approaches to identify the genetic drivers of FA. To succeed, these efforts need to be based on a consensus of how to diagnose FA.

**Table 1a: InFAC 2019 Statements regarding food allergy phenotypes for genetic research collaborations**

|   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|---|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 | <p>Initial collaborations should concentrate on peanut, hen's egg, and cow's milk allergy due to difficulties with other types of FA in regard to low prevalence and lack of consensus regarding diagnostic criteria (i.e.: 95% PPV cut-offs for allergen-specific IgE (sIgE) and skin prick test (SPT)) outside of DBPCFC.</p> <p>Statement 1 is made with the assumption that the underlying genetic model involves specific risk loci for specific foods. If one assumes that all FA are influenced by the same genetic risk loci, a study design grouping all FA may be more powerful, but would require DBPCFC for all cases that do not have well established diagnostic criteria outside of DBPCFC.</p>                                                                                                                                            |
| 2 | <p>Consider 4 tiers of FA diagnostic criteria, from most stringent to more moderate. These include, in order of decreasing stringency (Figure 1a):</p> <p>Tier 1: Convincing reaction confirmed by oral food challenge</p> <p>Tier 2: Convincing clinical history of a reaction with objective symptoms and confirmed exposure (Table 1b and below) <b>with</b> evidence of sensitization at or above 95% PPV cut-offs for SPT <b>OR</b> sIgE (Table 1c, Table S1, online supplement)</p> <p>Tier 3: Convincing clinical history of a reaction with objective symptoms and confirmed exposure (Table 1b), <b>with</b> any sensitization <math>\geq 3\text{mm}</math> SPT <b>OR</b> sIgE <math>\geq 0.35\text{kU}_\text{A}/\text{L}</math></p> <p>Tier 4: No convincing history <b>with</b> sensitization at or above 95% PPV cut-offs for SPT or sIgE</p> |

**Table 1b: Objective versus subjective symptoms occurring within 2 hours of ingestion of allergen**

| <b>System</b> | <b>Objective Symptoms</b>                              | <b>Subjective Symptoms</b>                                                          |
|---------------|--------------------------------------------------------|-------------------------------------------------------------------------------------|
| <b>Airway</b> | Angioedema of eyes, lips, tongue, oral cavity, pharynx | Isolated pruritus (skin, eyes, lips, tongue, throat, nose) with no visible symptoms |
|               | Dysphonia, hoarseness, laryngeal edema                 | Throat congestion                                                                   |
|               | Shortness of breath                                    |                                                                                     |
|               | Wheezing                                               | Chest tightness/pain                                                                |
|               | Coughing                                               |                                                                                     |

|                         |                                |                                                                 |
|-------------------------|--------------------------------|-----------------------------------------------------------------|
|                         | Sneezing episodes              |                                                                 |
|                         | Rhinorrhea                     | Nasal congestion                                                |
| <b>Cutaneous</b>        | Conjunctivitis, Tearing        | Isolated perioral redness                                       |
|                         | Erythema/flushing              | Skin discomfort                                                 |
|                         | Urticaria                      | Contact urticaria from food allergen contact, without ingestion |
| <b>Neurologic</b>       | Loss of consciousness/fainting | Dizziness, light-headedness                                     |
|                         | Seizures                       | Feeling of impending doom                                       |
| <b>Cardiac</b>          | Tachycardia                    |                                                                 |
|                         | Hypotension                    |                                                                 |
| <b>Gastrointestinal</b> | Emesis/Diarrhea                | Abdominal pain                                                  |
|                         |                                | Nausea                                                          |
| <b>Genitourinary</b>    |                                | Uterine cramping/contractions                                   |

Adapted from Pajno et al 2017, Allergy<sup>8</sup>

**Table 1c: Proposed 95% PPV cut-offs for skin prick test (SPT) and specific IgE (sIgE)**

| <b>Food type and age</b>     | <b>SPT size<br/>(mm)</b> | <b>sIgE levels<br/>(kU<sub>A</sub>/mL)</b> |
|------------------------------|--------------------------|--------------------------------------------|
| Peanut                       | ≥8                       | ≥14                                        |
| Peanut in ≤ 2 years          | ≥8                       | ≥14                                        |
| Cow's milk                   | ≥8                       | ≥15                                        |
| Cow's milk in ≤ 2 years      | ≥8                       | ≥5                                         |
| Hen's egg white              | ≥8                       | ≥7                                         |
| Hen's egg white in ≤ 2 years | ≥8                       | ≥2                                         |

Adapted from Nowak-Wegrzyn et al 2017<sup>9</sup>, see online supplement (Table S1)

Figure 1a: Proposed groups of food allergy phenotypes for genetic research

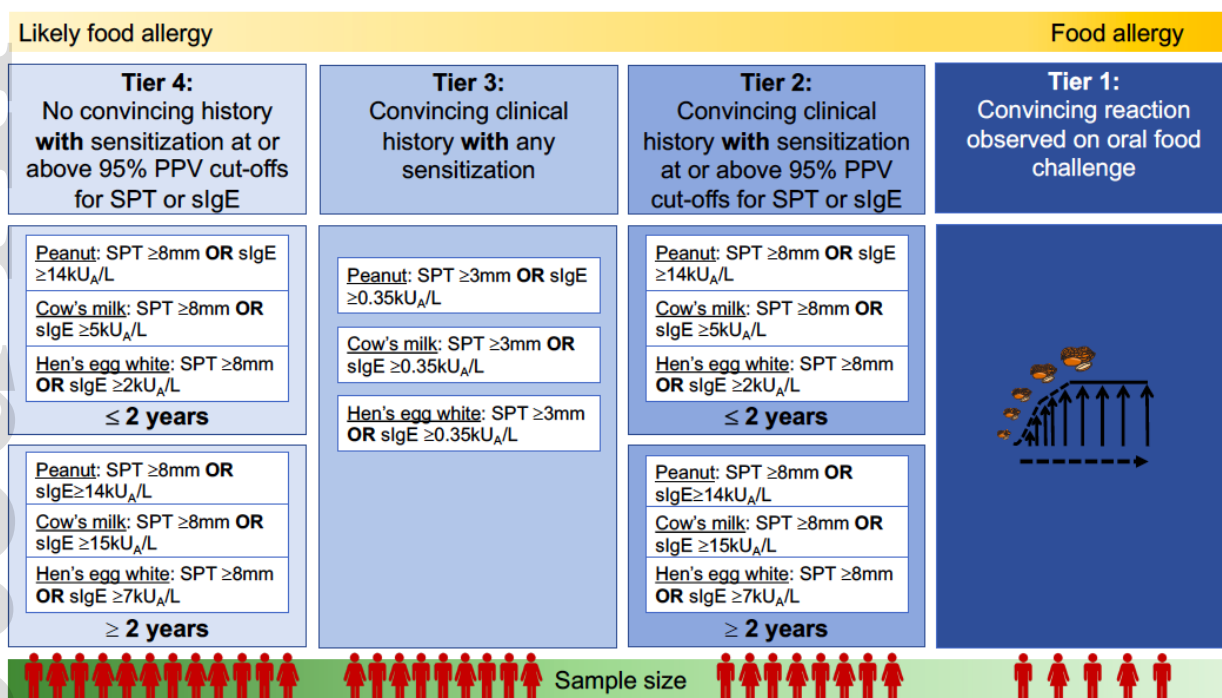
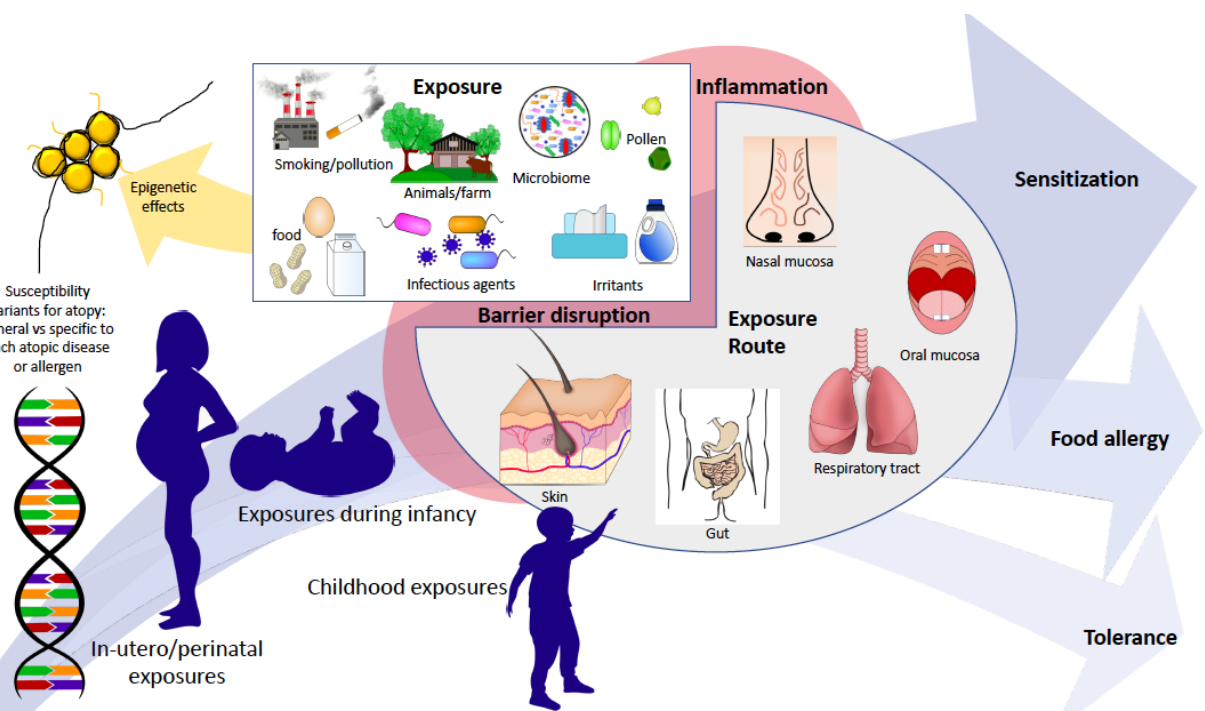


Figure 1b: Summary of factors involved in development of food allergy



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#### Statement of contribution:

YA, DD organized the InFAC meeting. YA, DM, TE, KN, GK, AC, EC, ES, CL, BM, IM, DR, SE, CH, JG, AE, LS, JH, MA, AS, DD participated in the InFAC meeting and contributed to the content and text of the paper. YA wrote the paper with valuable comments from DM, TE, KN, GK, AC, YL, EC, ES, CL, BM, IM, DR, SE, CH, JG, AE, LS, AS, DD.

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**Online supplementary material:**

**InFAC website:** <http://genapha.hli.ubc.ca/infac/>

**Additional references:**

**Table S1: Skin prick test and specific IgE cut-off references for peanut, cow's milk and hen's egg white**

| Reference               | Age                 | No. of Patients | SPT size (mm)                                            | sIgE levels (kU <sub>A</sub> /L) |
|-------------------------|---------------------|-----------------|----------------------------------------------------------|----------------------------------|
| <b>Peanut</b>           |                     |                 |                                                          |                                  |
| Bernard et al., 2003    | 1.5-16 years        | 96              | -                                                        | ≥5                               |
| Clark et al., 2003*     | Children and adults | 1000            | ≥8                                                       | ≥15                              |
| Dang, 2012              | 11-20 months        | 200             | -                                                        | ≥15                              |
| Hill et al., 2004       | Median 3 years      | 467             | ≥6 (≥2 years)<br>≥4 (<2 years)                           | -                                |
| Ho et al., 2006         | 4 months-19 years   | 680             | ≥8 (> 2 years)<br>≥4 (≤ 2 years)                         | -                                |
| Nicolaou et al., 2009   | ≤ 8 years           | 933             | ≥9                                                       | ≥45                              |
| Nolan et al., 2007      | 3.7-14.8 years      | 54              | ≥7**                                                     | -                                |
| Peters et al., 2013     | 11-15 months        | 438             | ≥8                                                       | ≥34                              |
| Rance et al., 2002      | 0.1-15.9 years      | 363             | ≥16                                                      | ≥57                              |
| Roberts et al., 2005    | 1.0-16.4 years      | 157             | ≥8                                                       | ≥15                              |
| Sampson, 2001           | 3 months-14 years   | 68              | -                                                        | ≥14                              |
| Sampson et al., 1997    | 0.6-17.9 years      | 196             | -                                                        | ≥15                              |
| Sporik et al., 2000     | Mean 3 years        | 467             | ≥8 (all children)<br>≥4 (< 2 years)                      | -                                |
| Wainstein et al., 2007  | 11 months-17 years  | 84              | ≥15                                                      | ≥10                              |
| <b>Cow's milk</b>       |                     |                 |                                                          |                                  |
| Garcia-Ara et al., 2001 | < 1 year            | 161             | -                                                        | ≥5                               |
| Hill et al., 2004       | Median 3 years      | 467             | ≥8 (≥2 years)<br>≥6 (<2 years)                           | -                                |
| Saarinen et al., 2001   | Mean 7 months       | 239             | ≥8 (92%PPV)                                              | ≥3.5 (94%)                       |
| Sampson, 2001           | 3 months-14 years   | 62              | -                                                        | ≥15                              |
| Sampson et al., 1997    | 0.6 to 17.9 years   | 196             | -                                                        | ≥32                              |
| Sporik et al., 2000     | Mean 3 years old    | 467             | ≥8 (all children)<br>≥6 (< 2 years)                      | -                                |
| Verstege et al., 2005   | 3 months-14.5 years | 385             | ≥12.5 (all children)<br>≥15.7 (1 year)<br>≥9.7 (<1 year) | -                                |

| <b>Hen's egg white</b>       |                     |     |                                                           |       |
|------------------------------|---------------------|-----|-----------------------------------------------------------|-------|
| Boyano-Martinez et al., 2002 | ≤ 2 years           | 58  | -                                                         | ≥3*** |
| Hill et al., 2004            | Median 3 years      | 467 | ≥6 (≥2 years)<br>≥5 (<2 years)                            | -     |
| Peters et al., 2013          | 11-15 months        | 652 | ≥4                                                        | ≥1.7  |
| Sampson, 2001                | 3 months-14 years   | 75  | -                                                         | ≥6    |
| Sampson et al., 1997         | 0.6 to 17.9 years   | 196 | -                                                         | ≥6    |
| Sporik et al. , 2000         | Mean 3 years old    | 467 | ≥7 (all children)<br>≥5 (<2 years)                        | -     |
| Verstege et al., 2005        | 3 months-14.5 years | 385 | ≥13.0 (all children)<br>≥13.3 (1 year)<br>≥11.2 (<1 year) | -     |

The table shows cut-off of 95% PPV or above.

\* Results are based on mixed peanuts and tree nuts.

\*\* Value is based on 93% PPV. No higher PPV is reported in the article.

\*\*\* Value is based on 93% PPV, and interpreted as such from Caubet & Wang, 2011.

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**Table S2: Astier score for reaction severity:** (Astier C, Morisset M, Roitel O, Codreanu F, Jacquenet S, et al. (2006) Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. J Allergy Clin Immunol 118: 250–256.)

| Symptom score | Symptoms                                                                                                                                                                    |
|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>0</b>      | No symptoms                                                                                                                                                                 |
| <b>1</b>      | Abdominal pain that resolved without medical treatment, rhinoconjunctivitis or urticaria < 10 papules, rash                                                                 |
| <b>2</b>      | <b>One organ involved</b> , *abdominal pain requiring treatment, *generalized urticaria, *non-laryngeal angioedema, *mild asthma (cough, fall of peak expiratory flow <20%) |
| <b>3</b>      | <b>Two organs involved</b> (of symptoms mentioned under 2)                                                                                                                  |
| <b>4</b>      | <b>Three organs involved</b> (of symptoms mentioned under 2) <b>or asthma</b> requiring treatment or laryngeal edema, or hypotension                                        |
| <b>5</b>      | <b>cardiac and respiratory symptoms requiring hospitalization in the intensive care unit</b>                                                                                |

**World Allergy Organization definition of Anaphylaxis** (Simons FE, Arduzzo LR, Bilo MB, et al. World allergy organization guidelines for the assessment and management of anaphylaxis. The World Allergy Organization journal. 2011;4(2):13-37.)

Anaphylaxis is highly likely when any one of the following three criteria is fulfilled:

- 1) Sudden onset of an illness (minutes to several hours), with involvement of the skin, mucosal tissue, or both (e.g. generalized hives, itching or flushing, swollen lips-tongue-uvula) and at least one of the following:
  - a. Sudden respiratory symptoms and signs (e.g. shortness of breath, wheeze, cough, stridor, hypoxemia)
  - b. Sudden reduced blood pressure or symptoms of end-organ dysfunction (e.g. hypotonia [collapse], incontinence)
- 2) Two or more of the following that occur suddenly after exposure to a likely allergen or other trigger for that patient (minutes to several hours):
  - a. Sudden skin or mucosal symptoms and signs (e.g. generalized hives, itch-flush, swollen lips-tongue-uvula)
  - b. Sudden respiratory symptoms and signs (e.g. shortness of breath, wheeze, cough, stridor, hypoxemia)
  - c. Sudden reduced blood pressure or symptoms of end-organ dysfunction (e.g. hypotonia [collapse], incontinence)
  - d. Sudden gastrointestinal symptoms (e.g. crampy abdominal pain, vomiting)
- 3) Reduced blood pressure after exposure to a known allergen for that patient (minutes to several hours)

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