TABLE 5-2 Continued

Guideline (reference)	Year	Country	Breastfeeding
American Academy of Pediatrics (Greer et al., 2008)	2008	US	For infants at high risk of developing atopic disease, evidence suggests that exclusive breastfeeding for at least 4 months compared with feeding intact cow milk protein formula decreases the cumulative incidence of atopic dermatitis and cow milk allergy in the first 2 years of life.

Early Introduction of Foods	Infant Formula	Diet of Mother	Prebiotics or Probiotics
Although solid foods should not be introduced before 4 to 6 months of age, there is no current convincing evidence that delaying their introduction beyond this period has a significant protective effect on the development of atopic disease regardless of whether infants are fed cow milk protein formula or human milk. This includes delaying the introduction of foods that are considered to be highly allergic, such as fish, eggs, and foods containing peanut protein. For infants after 4 to 6 months of age, there are insufficient data to support a protective effect of any dietary intervention for the development of atopic disease.	In studies of infants at high risk of developing atopic disease who are not breastfed exclusively for 4 to 6 months or are formula fed, there is modest evidence that atopic dermatitis may be delayed or prevented by the use of extensively or partially hydrolyzed formulas, compared with cow milk formula, in early childhood. Extensively hydrolyzed formulas may be more effective than partially hydrolyzed in the prevention of atopic disease. There is no convincing evidence for the use of soy-based infant formula for the purpose of allergy prevention.	Current evidence does not support a major role for maternal dietary restrictions during pregnancy or lactation.	

continued

TABLE 5-2 Continued

Guideline (reference)	Year	Country	Breastfeeding
National Health Service (NHS, 2015b)	2015	UK	Breast milk or first infant formula for first 6 months.
National Health and Medical Research Council (NHMRC, 2013)	2012	Australia	Exclusive breastfeeding until around 6 months of age. For infants with a family history of allergy, continue breastfeeding while introducing solid foods.

Early Introduction of Foods	Infant Formula	Diet of Mother	Prebiotics or Probiotics
Introduce cow milk, eggs, wheat, gluten, nuts, peanuts, peanut products, seeds, fish and shellfish one at a time and not before 6 months.	Infant formula made from cow or goat milk is the only suitable alternative to breast milk in the first 12 months. Only use soy-based infant formula if advised by health care provider. Follow-on milks are available for babies older than 6 months, but there is no need to change over to these. If child has an allergy or intolerance to milk, health care provider can advise on suitable milk alternatives.		
For infants with a family history of allergy, solid foods should be introduced at about 6 months of age.	If breastfeeding is discontinued for any reason, there is no advantage in using special formulas, except under medical supervision. Soy-based formulas do not prevent or reduce the risk of developing allergies and are not a suitable alternative to cow milk–based formulas.	Dietary elimination of potential allergens during pregnancy is not recommended for preventing childhood allergy.	The evidence on probiotics or prebiotics in infant formula to prevent atopic disease varies.

continued

TABLE 5-2 Continued

Guideline (reference)	Year	Country	Breastfeeding
Australasian Society of Clinical Immunology and Allergy (ASCIA, 2016a,b)	2016	Australia	Breastfeeding is recommended for at least 6 months.[b]
Academy of Nutrition and Dietetics (AND, 2015)	2015	US	Exclusive breastfeeding provides optimal nutrition and health protection for the first 6 months of life and breastfeeding with complementary foods from 6 months until at least 12 months of age is the ideal feeding pattern for infants. Breastfeeding should be supported and preserved even under adverse or challenging conditions, such as prematurity, allergies, chronic illness, and multiple births.

NOTE: UK = United Kingdom; US = United States.

[a] Australasian Society of Clinical Immunology and Allergy, Canadian Society of Allergy and Clinical Immunology, European Academy of Allergy & Clinical Immunology, Israel Association of Allergy and Clinical Immunology, Japanese Society for Allergology, Society for Pediatric Dermatology, and World Allergy Organization.

[b] For all infants (not as a prevention for allergic diseases).

Early Introduction of Foods	Infant Formula	Diet of Mother	Prebiotics or Probiotics
Recommends the introduction of complementary "solid" foods within the window of 4-6 months and preferably while breastfeeding, regardless of whether the food is considered to be a common food allergen.	In children with confirmed cow milk and soy allergy, appropriate formula is available on prescription. There is no consistent convincing evidence to support a protective role for partially hydrolysed formulas or extensively hydrolyzed formulas for the prevention of food allergy in infants or children.	Exclusion of any particular foods (including foods considered to be highly allergenic) from the maternal diet during pregnancy or breastfeeding is not recommended.	Recommendations about probiotic supplements cannot currently be made.

TABLE 5-3 Evidence-Based Review Grading System Used by the Committee to Evaluate the Association Between Potential Risk Determinants and Food Allergies

Strong	The conclusion statement is substantiated by a large, high quality, and/or consistent body of evidence that directly addresses the question. There is a high level of certainty that the conclusion is generalizable to the population of interest, and it is unlikely to change if new evidence emerges.
Moderate	The conclusion statement is substantiated by sufficient evidence, but the level of certainty is restricted by limitations in the evidence, such as the amount of evidence available, inconsistencies in findings, or methodological or generalizability concerns. If new evidence emerges, there could be modifications to the conclusion statement.
Limited	The conclusion statement is substantiated by insufficient evidence, and the level of certainty is seriously restricted by limitations in the evidence, such as the amount of evidence available, inconsistencies in findings, or methodological or generalizability concerns. If new evidence emerges, there could likely be modifications to the conclusion statement.
Grade not assignable	A conclusion statement cannot be drawn due to a lack of evidence, or the availability of evidence has serious methodological concerns.

SOURCE: DGAC, 2015.

sensitization to the same food measured by sIgE and/or a positive skin prick test (SPT) to this specified food, not by OFC.

Overall, evidence exists of genetic predisposition for food allergy based on family aggregation (Tsai et al., 2009) and heritability studies (Liu et al., 2009; Sicherer et al., 2000), the latter showing a wide range of values between 0.15 and 0.88. However, as with other complex diseases that are polygenic, challenges remain to identify what contribute to the "missing heritability."

The committee concludes that although some evidence from various lines of investigation suggests that genetics contribute to the development of food allergies, none of the studies on the association of food allergy with specific loci examined to date has provided conclusive and consistent findings across populations.

Interaction Between Genetics and Environment: Migration Studies

As mentioned above, environmental exposures, including lifestyle and diet, interact with genetic predisposition to modify the risk of disease. The

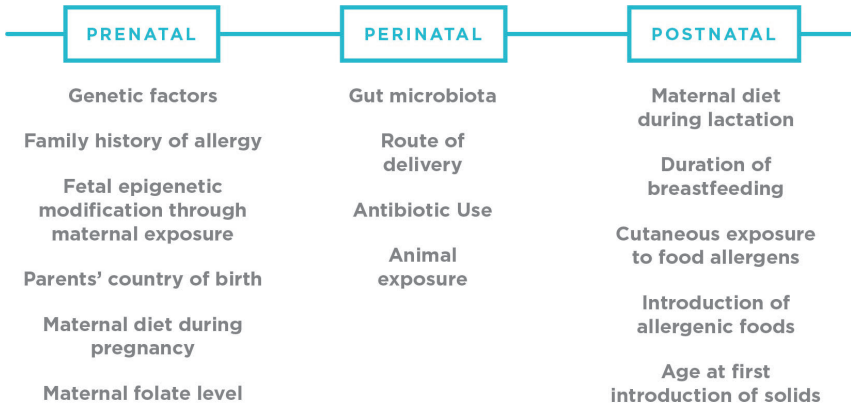

FIGURE 5-2 Major genetic and environmental determinants of food allergy risk.

"natural experiment" of migration has provided an opportunity to postulate a possible protective effect of the Asian environment on Asian children that is removed on migration to a developed country such as the United States or Australia, where risk of allergic disease rises. In HealthNuts, challenge-confirmed peanut allergy was about three times more common in infants whose parents were born in East Asia compared to those with parents born in Australia (Koplin et al., 2014). Similar effects were seen for other food sensitizations and food allergies and for eczema. This increased risk appears to have occurred in a single generation and to be specific to infants of Asian parents. This effect was not seen among infants whose parents were born in the United Kingdom or Europe.

More recently in a large cohort of more than 65,000 children whose parents undertook a survey as their children entered primary school (age 5 years), the finding of increased rates of nut allergy in Asian children born in Australia was replicated. However the most intriguing observation in this study was the finding that children born in Asia who subsequently migrated to Australia before the age of 5 years were protected from the development of food allergy (Panjari et al., 2016).

Migration may be associated with changes to a number of factors (some of which were not measured in HealthNuts) that might be inter-related (Allen and Koplin, 2015) (see Figure 5-3). These include humidity (and its impact on skin barrier function), microbial exposure (hygiene hypothesis), and dietary changes and changes in latitude (vitamin D). For example, changes to the skin barrier function and risk of eczema as an early risk factor of food allergy may result from higher humidity in Asia than Australia

TABLE 5-4 Summary of Studies Associating Specific Genes with Food Allergy

Author, Year	Study Design, Country	Population	N	Candidate Gene	Outcome	Summary
Senechal et al., 1999	Observational, Europe	European-born white adults	42 atopic 42 healthy	HLA	Apple allergy	Association with the HLA-DRB1*07 allele
Hand et al., 2004	Observational, UK	Allergy clinic patients: ages 3-56 years; 81 white 3 mixed race 40 male 44 female Atopic controls: ages 16-61 years; 31 male 51 female	84 nut-allergic patients 82 atopic non-nut-allergic patients 1,798 random blood donors	HLA	Nut allergy (peanut, Brazil nut, hazelnut, walnut, cashew, almond, and pecan)	Increased for HLA-beta*07 and HLA-DRB1*11, HLA-DRB*13, and HLA-DQB1*06 alleles
Madore et al., 2013	Observational, Canada	Peanut-allergic Caucasian children, mean age 11 years Controls: mean age 4 years	590 cases 332 controls	HLA	Peanut allergy	HLA-DQB1*02 and HLA-DQB1*06:03P associated with peanut allergy
Hong et al., 2015	Observational, US	Participants in the Chicago Food Allergy Study	1,315 children 1,444 parents	HLA	Peanut allergy	HLA-DR and -DQ gene region at 6p21.32, tagged by rs7192 and rs9275596

Woo et al., 2003	Observational, US	Food allergic patients, mean age 5.2 years, 74% male, 83% white Non-atopic, non-asthmatic adult controls	77 cases 61 controls	CD14	Food allergy	The C-159T SNP associated with food allergy
Campos et al., 2007	Observational, Japan	Food-allergic children, mean age 7.1 years Non-food-allergic controls, mean age 9.45 years	88 cases 101 controls	CD14	Food Allergy	No association with the C-159T or the C-550T
Torgerson et al., 2007	Case series, France	Index case with IPEX syndrome and other family members	11	FOXP3	Severe food allergy	1300-bp deletion could cause severe food allergy
Siegel et al., 2013	Observational, US	Atopic patients: 40% female, mean age 14.8 years Controls: 61% female, mean age 34.5 years	65 patients with severe atopic disease 41 healthy controls	STAT3	Food allergies (egg, milk, or peanut)	Complex association between this locus and allergic phenotypes

continued

TABLE 5-4 Continued

Author, Year	Study Design, Country	Population	N	Candidate Gene	Outcome	Summary
Amoli et al., 2002	Observational, UK	Nut-allergic, Caucasian patients, mean age 10 years Healthy atopic, non-allergic controls UK Caucasian blood donors	71 patients 45 controls 184 blood donors	STAT6	Nut-allergy (peanut, cashew, Brazil nut, pecan, almond, hazelnut, or walnut)	The G allele at the G2964A SNP increased in nut-allergic patients.
Negoro et al., 2006	Observational, Japan	Allergic children	220	STAT6	Food allergy	No association of G2964A and severity of food allergy
Kusunoki et al., 2005	Observational, Japan	Children with atopic dermatitis, >5 years of age	118	SPINK5	Food allergy	The 1258AA or 1258AG carriers have higher prevalence of food allergy
Negoro et al., 2006	Observational, Japan	Allergic children, mean age 7.3 years	220	IL10	Food allergy	No association with the C-627A SNP
Campos et al., 2008	Observational, Japan	Food-allergic children, mean age 7.6 years, 63% male Atopic control children without food allergy, mean age 8.2 years, 64% male	111 cases 115 controls	IL10	Food allergy	No association with the C-627A SNP; but the -1082AA genotype was associated with higher risk

Chen et al., 2012	Observational, Taiwan	Food-allergic patients, age range 1-32 years; 62% male Non-food-allergic controls, age range 1-59 years, 40% male	37 cases 52 controls	IL10	Food allergy	Both the -1082A/G and the -592A/C SNPs were associated with food allergies
Liu et al., 2004	Observational, Germany	German children who participated in the German Multicenter Allergy Study	823	IL13	Food sensitization	C-1055T higher risk
Gaudieri et al., 2012	Observational, Australia	Children recruited antenatally from healthy pregnant mothers; followed from birth to age 5 years	35 allergic 35 non-allergic	IL28B	Food allergy	The rs12979860 SNP associated positively with food allergy
Venkataraman et al., 2014	Observational, UK	Isle of Wight birth cohort; children ages 1-18 years	1,456	FLG	Food allergy	FLG LOF mutations associated with food allergy.
Tan et al., 2012a	Observational, Australia	HealthNuts Cohort study participants; white infants, age 1 year	700	FLG	Food sensitization/ allergy	FLG LOF mutations do not increase the risk of food allergies beyond that of food sensitization

continued

TABLE 5-4 Continued

Author, Year	Study Design, Country	Population	N	Candidate Gene	Outcome	Summary
Brough et al., 2014	Observational, UK	Birth cohort of the Manchester Asthma and Allergy Study, children ages 1-11 years	1,184	FLG	Peanut allergy	Positive association with peanut allergy
Li et al., 2012	Observational, China	Atopic dermatitis outpatients, mean age 3.5 years, 64.3% male	249	FLG	Food sensitization	Interaction of K4671X mutation and the combined mutations in FLG related to sensitization to peanut allergens in patients with atopic dermatitis
Oxelius et al., 2015	Observational, Germany	Children from the German Multicenter Allergy Study, Caucasian, age 1 year or 10 years	194	IGHG genes	Food sensitization	The IGHG*bfn haplotype (B*bfn cells) and increased innate IgG2*n levels are predictive factors for IgE food sensitization in childhood

NOTE: FLG = filaggrin; HLA = human leukocyte antigen; IGHG = immunoglobulin heavy locus gene; IL = interleukin; LOF = loss of function; UK = United Kingdom; US = United States.

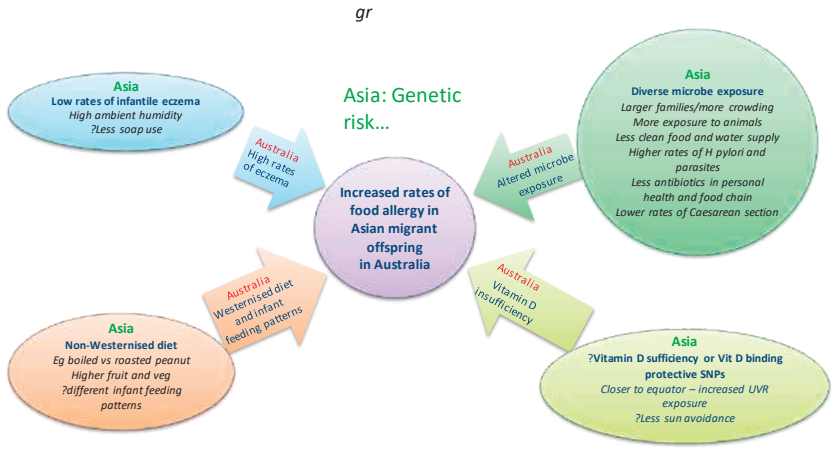

FIGURE 5-3 Modifiable lifestyle risk factors that could explain the rise in food allergy risk in offspring of Asian migrants in Australia.
NOTE: SNP = single nucleotide polymorphism; UVR = ultraviolet radiation.
SOURCE: Allen and Koplin, 2015. Reprinted with permission from Elsevier.

but equally may result from differences in infant washing practices (types of soap and water composition) that occur in each country and may exert an effect through the hygiene hypothesis. Microbial exposure factors that differ not only include variations in the quality of water supply (and differences in risk of waterborne gastrointestinal infections) but also differences in microbes that are a part of the food chain supply (for example, in unwashed vegetables or higher use of antibiotics in the food chain supply of meat-producing animals), number of children in a family, and issues of crowding and exposure to pets, farm animals, and stray animals (which may have higher rates of parasites), and variations in overprescribing of antibiotics in each region. Dietary differences are multiple (e.g., higher use of herbicides and pesticides that might affect the microbial load of food and increased sterilization; use of plastic in developed countries; cooking practices that may alter the allergenicity of food; different vitamin D status).

Epigenetics

The contribution of epigenetics has been more extensively studied for other allergic diseases, including asthma, eczema, and allergic rhinitis, as reviewed by Hong and Wang (2014), than for food allergies. In light of the

atopic march and common comorbidities between food allergies and these other allergic diseases, one may speculate that a link between epigenetic changes and the development of food allergies is possible, but at this time the evidence is quite limited and comes from indirect studies such as the migration studies described above.

Direct evidence to establish the relevance of epigenetic changes as a mediator of genetic susceptibility to food allergies is very limited. The most up-to-date knowledge about the role of epigenetics in food allergy has been summarized in a recent review by Neeland et al. (2015). In their epigenome-wide association study (EWAS) of food allergies,[8] Martino et al. measured genome-wide DNA methylation profiles from CD4+ T-cells (see Chapter 2) on a birth cohort of 12 children with IgE-mediated food allergy diagnosed at 12 months; 12 individuals with no food allergies were controls (Martino et al., 2014). A number of statistically significant differentially methylated probes (DMPs) were identified from DNA obtained from samples taken at birth and at 12 months of age. Of interest is the finding of 96 allergy-associated non-SNP DMPs that were present at time of birth before the expression of the disease. These could be causally related to its expression, including several mitogen-activated protein kinase (MAPK) signaling molecules. Therefore, the authors concluded that "dysregulation of DNA methylation at MAPK signaling-associated genes during early CD4+ T-cell development may contribute to suboptimal T-lymphocyte responses in early childhood" that could influence the development of food allergy (Martino et al., 2014). However, this is a small study and, therefore, its findings need independent validation in larger studies and other populations.

Support for a role of epigenetics in food allergies is provided by the results from a food allergy GWAS carried out in 2,759 U.S. participants (1,315 children and 1,444 parents) from the Chicago Food Allergy Study (Hong et al., 2015). In a recent study in the Chicago cohort, Hong et al. conducted an EWAS of cow milk allergy using a two-stage approach (Hong et al., 2016). During the discovery stage, DNA methylation was measured at 485,512 genomic loci in whole blood samples from 106 Caucasian children with cow milk allergy (cases) and 76 nonallergic and nonatopic Caucasian children (controls) using the Illumina HumanMethylation450 arrays. The findings were confirmed in a small replication sample (5 cases and 20 controls). The researchers demonstrated that altered DNA methylation in genes involved in the Th1-Th2 pathways and some novel candidate genes are associated with cow milk allergy.

[8] Epigenome-wide association studies (EWASs) are large-scale, systematic studies that explore the association between the epigenetic variations and diseases, equivalent to genome-wide association studies (GWASs).

The committee concludes that relative to other immune-related diseases, only a few studies have been conducted that directly support a contribution of epigenetic factors to the development of food allergies. Limited evidence from ecological studies and studies on methylation signatures of participants with food allergies suggest that gene-environment interactions and underlying epigenetic mechanisms need to be taken into account when exploring potential pre- and postnatal risk factors for food allergy.

ENVIRONMENTAL RISK FACTORS

Microbial Exposure Hypotheses

As mentioned in Box 5-1, evidence increasingly suggests that the interaction between the host microbiome and the immune system is essential to the development of immune regulation and oral tolerance (Martin et al., 2010). Exposure to microbes after birth prompts the maturation of the mucosal immune system (Kelly et al., 2007). The composition and timing of exposure to gut microbiota, and their possible role in disease development or prevention have been considered as explanations for the development of food allergy (Li et al., 2014; McLoughlin and Mills, 2011; Prince et al., 2015). The microbial hypothesis proposes that a decrease in early childhood exposure to microbes or their products may hinder the normal development of early immunoregulatory responses. This leaves the immune system more susceptible to inappropriate reactivity to innocuous antigens, resulting in the development of "allergic" diseases.

The overall microbial hypothesis encompasses two different concepts—the "Hygiene Hypothesis" and the "Old Friends Hypothesis." The Hygiene Hypothesis, originally explained in the landmark paper by David Strachan in 1989, described a protective effect of an increasing number of siblings in a household on the risk of developing allergic rhinitis (Strachan, 1989). This was thought to potentially relate to the shared exposure to common childhood infections transmitted through direct contact with older siblings or by maternal contact with her older children prenatally. Although a protective sibling effect has been confirmed for challenge-proven food allergy outcomes (Koplin et al., 2012a) and for various food sensitization and allergy outcomes (Marrs et al., 2013), the mechanism(s) underlying this phenomenon is not clear. Although the finding is interesting and reproducible, changes to postwar houses and sanitation, and sizes of families, as well as the emergence of national immunization programs with high uptakes, also should be considered in attempting to identify the mechanisms underlying the protective effects of siblings.

Second, evidence of a protective effect of dog ownership on food allergy risk may point to the benefit of sharing of microbes or even parasites, the

latter underpinning the idea of the Old Friends hypothesis. Although this hypothesis was predicated on the assumption that IgE antibody–associated immune responses developed in part as a mechanism of host defense against parasite infestation, recent evidence indicates that, at least in mice, IgE antibody–associated immune response also can confer increased acquired resistance to the morbidity and mortality induced by arthropod and reptile venoms (Galli et al., 2016; Marichal et al., 2013; Palm et al., 2013; Starkl et al., 2016). Speaking more broadly, early evidence suggests a difference between the prevalence of food allergy in rural versus urban environments that appears to be reflected in rising rates of food allergy described in cities in China undergoing rapid urbanization (Hu et. al., 2010).

The main environmental factors contributing to the microbial exposure hypothesis include route of delivery at birth, antibiotic use, exposure to pets/animals, and immunization. Breastfeeding has been linked to infant immune development (Praveen et al., 2015) and the composition of the microbiota (Azad et al., 2016). It would therefore be plausible that a mechanism linking food allergy risk and breastfeeding could be mediated through microbiome modulation (Fooladi et al., 2013). However, no published studies to date have investigated this hypothesis, and the data linking breastfeeding and food allergy are inconclusive, potentially due to reverse causality and the inability to randomize infants for breast- versus formula-feeding. Therefore breastfeeding will not be included in this section; instead the effect of breastfeeding is included as part of the "Allergen Avoidance Hypothesis" (see p. 185). The ingestion of prebiotics and probiotics could modify the gut microbiota in a way to change immune system functionality and atopic diseases. Therefore, their use as potential risk factor for food allergies also is included.

A systematic review of the evidence linking microbial exposure and food allergy was published by Marrs et al. (Marrs et al., 2013). The authors reviewed scientific publications available in Medline between 1948 and July 2012. The key findings of this review will be briefly summarized below, but the focus will be on reviewing the findings of papers published since July 2012.

Gut Microbiota and the Use of Probiotics and Prebiotics

Data on microbial profiling and its relationship to disease are still not sufficiently detailed to consider specific microbiota modifications as a food allergy prevention strategy. However, some emerging data suggest that changes in microbiota could influence food allergies, offering further support for the microbial exposure hypothesis (West et al., 2015).

Marrs et al. included five studies in their systematic review that investigated characteristics of gut microbiota, two of which used food challenge

outcomes and three that used food sensitization parameters (Marrs et al., 2013). The two manuscripts that ranked highest in quality and measured food allergy were from the same study of Spanish infants who were diagnosed with IgE-mediated cow milk allergy by milk challenge at a tertiary referral center. Differences in microbiota were identified but unfortunately none of the results was adjusted for diet. The Marrs review also included 11 RCTs in which microbial supplementation was the intervention as a potential prevention or treatment of food allergies or sensitization. Although the quality varied, the two highest quality studies that measured food allergy by OFC to assess whether microbial supplementation may be used to prevent or treat food allergies or sensitization found no benefit.

More recent data originate from the Canadian Synergy in Microbiota (SyMBIOTA) study, part of a larger Canadian research effort on the microbiota. This large 6-year longitudinal study is using metadata and samples from the Canadian Healthy Infant Longitudinal cohort to discern relationships between infant fecal microbiota and each of a group of factors, including antibiotic use, pets, and food sensitization (Kozyrskyj, 2015). Their data suggest that lower species richness in microbiota of infants (N=166, ages 3 and 12 months) might be a predictor of food (i.e., for egg, milk, and peanut) sensitization (SPT at age 12 months), even when adjusting for birth delivery mode, antibiotic use, or breastfeeding (Azad et al., 2015). Their research also revealed that sensitization occurred after the changes in microbiota diversity and richness, two commonly used indexes. Therefore, this ratio could potentially be used as a predictor of food sensitization, a potential surrogate for food allergies. Each quartile increase in richness at 3 months was associated with a 55 percent reduction in risk for food sensitization by 1 year (adjusted odds ratio [aOR] 0.45; 95% confidence interval [CI]: 0.23-0.87).

One meta-analysis of 10 RCTs (Kong et al., 2014) reported no significant difference in the incidence of food allergies comparing prenatal and postnatal probiotics supplementation with placebo or control. However, the food allergy assessments were not described in the meta-analysis. The World Allergy Organization (WAO) has recently conducted a systematic review on the relationship between supplementing the diet of pregnant or lactating women or infants with probiotics and allergy diseases. Six trials explored the relationship with food allergies but none of them made the direct comparison of probiotics versus no probiotics in pregnant women or in breastfeeding women for prevention of allergy in their children. None of the trials found differences in food allergy with probiotic supplementation (Cuello-Garcia et al., 2015). Two additional observational studies found during the committees' evidence-based search did not find an association between the addition of probiotics to infants' diets (Loo et al., 2014; West et al., 2013). The most recent work on the effect of prebiotics in

food allergy, also conducted by the WAO (Cuello-Garcia et al., 2016), is a guideline that seems to be based on a systematic review. The methods of systematic review, however, were not fully reported and no other source or citation to the systematic review was found. The guideline is based on studies investigating the relationship between prebiotics consumption by women during pregnancy or lactation and by healthy infants for preventing various allergic symptoms, including food allergy. Only one intervention study assessed the risk of developing food allergy in infants consuming an infant formula containing oligosacharides (Ivakhnenko and Nyankovskyy, 2013). That study (N=240) found that infants who had been fed with breast milk or oligosaccharide-supplemented infant formula had significantly fewer allergic reactions to food products compared to the infants fed the standard formula (3.92 percent and 4.84 percent versus 16.98 percent, respectively; $P<0.05$).

The committee concludes that, at this time, only a few studies have been conducted on the relationship between changes in the microbiota and food sensitization and, therefore, the evidence supporting this relationship is limited. RCTs on probiotic and prebiotics supplementation are few and have methodological limitations. Therefore, the committee concludes that the evidence is limited and does not yet support a decrease in food allergy risk from the use of probiotics or prebiotics by pregnant and lactating women or by infants. Additional research would be needed before recommending the use of prebiotics or probiotics to prevent the onset of food allergies.

Route of Delivery

The composition of the gut microbiota is influenced by route of delivery. Vaginally-delivered infants harbor bacterial communities resembling their mother's vaginal microbiota. In contrast, infants delivered by cesarean section have bacterial communities similar to those found on the skin surface (Dominguez-Bello et al., 2010). In light of the fact that the gut microbiome plays a central role in the development of immune regulation and oral tolerance, it is not surprising that investigators have examined the question of whether caesarean delivery increased the risk of food allergy.

In their systematic review, Marrs et al. identified 13 publications. Of these, five identified food allergy through OFCs. All 13 publications, except for the study of lowest quality, reported an increased risk of developing food allergy or food sensitization in children delivered by cesarean section (Marrs et al., 2013). Six of these associations were significant. However, only two included clinical food allergy diagnoses. Of the studies included for review, these two studies yielded the highest quality data. The studies used 2,803 consecutive mother-infant pairs from a Norwegian birth

cohort surveyed at 12, 18, and 24 months. When children were challenged with food orally using open or double-blind protocols, cesarean section was associated with a significantly higher risk for cow milk allergy. This occurred only in the subgroup of children with atopic mothers, however (aOR: 9.6 [95% CI: 1.8-52.4]) (Eggesbo et al., 2005). They also observed a nonsignificant 60 percent increase in egg allergy risk up to age 2 years (Eggesbo et al., 2003).

The Marrs review also included a prospective nested case-control study of 16,237 infants in Finland, ages 0 to 2 years (Metsala et al., 2010). Infants whose parents had received a reimbursement for the cost of specialized formula based on diagnosis of cow milk allergy were recruited, and the allergy was certified by a pediatrician using clinical exam, symptoms, elimination diet, SPT, and elevated sIgE or open challenge test (Metsala et al., 2010). Controls were randomly selected infants who were matched for age, sex, and delivery hospital. A significant relationship between cesarean delivery and cow milk allergy was observed (aOR: 1.18; 95% CI: 1.10-1.27).

Lodge et al. conducted a more recent review of systematic reviews and found two systematic reviews that included six original studies (Lodge et al., 2013). An association between cesarean section delivery and increase in food allergy is seen in only the three smallest studies. Two of these studies used specific IgE to food allergens as the outcome measurement. No conclusion was reached by the authors due to methodological flaws (i.e., small size studies or inaccurate food allergy measurement).

Since the Marrs' systematic review, six prospective cohort studies investigating associations between cesarean delivery and allergy risk have been published. They include studies conducted in Australia (Peters et al., 2014), France (Pele et al., 2013), the United Kingdom (Grimshaw et al., 2014), the United States (Luccioli et al., 2014; McGowan et al., 2015), and a five-country study (Depner et al., 2013) totaling 25,688 cases and controls. Overall, these studies found no significant associations between cesarean delivery and a variety of food allergies. The age of the children in the studies ranged from 0 to 5 years, and most included physician-diagnosed food allergy. Minimum criteria for diagnosis were sIgE to food allergen or a positive SPT. However, Luccioli et al. used physician diagnosis based on parental report (Luccioli et al., 2014). The largest study was the Australian HealthNuts Study (Peters et al., 2014), which recruited 5,276 infants at immunization clinics. These infants (2,848 of the total recruited) were investigated for open challenge-proven egg, peanut, and sesame allergy. However, no significant association was demonstrated with mode of delivery (Peters et al., 2014). Two retrospective case-control studies from Finland (N=3,181) (Pyrhonen et al., 2013) and the United States (N=291) (Dowhower Karpa et al., 2012) also did not show an association between cesarean delivery and food allergy.

The variation in association between mode of delivery and risk of food allergy may be partly explained by the fact that some studies have been unable to distinguish between whether cesarean delivery had been done on an elective or emergency basis (e.g., Koplin et al., 2012a; Peters et al., 2014). Emergency cesarean delivery is generally associated with rupture of membranes. As a result, the baby has some exposure to vaginal commensal bacteria during labor. However, the exposure is not usually to the same extent as vaginal delivery. However, because the proportion of emergency cesarean deliveries is usually relatively small compared to elective cesarean deliveries, we would still expect to see some association between mode of delivery and food allergy. This would be true even in those studies that could not differentiate emergency from elective cesarean deliveries, particularly in the larger and better powered studies. It also should be noted that the association between cesarean delivery and allergic risk could be misinterpreted due to the potential for reverse causation similar to breastfeeding.

Only a few observational studies have been conducted on the relationship between food sensitization or food allergy and cesarean delivery. The studies have methodological limitations. Therefore, the committee concludes that, at this time, evidence to support an increased risk for food sensitization or food allergy due to giving birth by cesarean delivery is limited. Strong evidence is unlikely to be forthcoming because of the ethical inability to randomize a population to deliver a baby by cesarean section. However, additional prospective research studies are needed.

Antibiotic Use

Antibiotics are known to cause short-term and, in some cases, lasting alterations in the microbiota (Faa et al., 2013). Infants can be exposed to antibiotics pre-, peri-, or postnatally as individual exposures or multiple exposures across this time, when the microbiome is not well established and is more susceptible to perturbations. The Marrs et al. systematic review reported no relationship between antenatal or postnatal antibiotic exposure and increased risk of food allergy (Marrs et al., 2013).

Since 2012, two prospective cohort studies of food allergic children have been published that were not included in the Marrs systematic review (Marrs et al., 2013). Studies in Finland (Metsala et al., 2013) and the United Kingdom (Grimshaw et al., 2014) and one retrospective case control study from the United States (Dowhower-Karpa et al., 2012) investigated associations between antibiotic exposure and food allergy risk. In those infants whose mother used antibiotics before or during pregnancy, respectively, the Finnish prospective, nested case-control study (N=16,237) reported a statistically significant 26 percent (aOR: 1.26; 95% CI: 1.20-1.33) and 21

percent (aOR: 1.21; 95% CI: 1.14-1.28) increased risk for cow milk allergy (determined by OFC) (Metsala et al., 2013). An even greater risk of cow milk allergy (aOR: 1.71; 95% CI: 1.59-1.84) was reported in infants who were treated with antibiotics between birth and 1 month of age (Metsala et al., 2013).

However, two other studies described below showed no statistically significant association. Cases (N=41) and controls (N=82) in the UK study were drawn from the Prevalence of Infant Food Allergy (PIFA) study (Grimshaw et al., 2014). Children in this study were part of the larger EuroPrevall birth cohort. Food allergy was diagnosed using SPT, physical exam, clinical history, sIgE, and DBPCOFC. Maternal antibiotic use during or after pregnancy or during breastfeeding was not associated with increased risk of food allergy in the infant. However, administration of the antibiotic to the infant was not assessed (Grimshaw et al., 2014). In a retrospective case (N=99) control (N=192) design, Dowhower Karpa et al. found no association between peripartum or neonatal antibiotic exposure and food allergy, diagnosed by positive sIgE or SPT (Dowhower Karpa et al., 2012).

Thus, taking together the results of the Marrs systematic review (Marrs et al., 2013) and the three studies published since, only one study (Metsala et al., 2013) has reported a link between antibiotic use and food allergy. The strengths of that study is the large sample size (more than 16,000 children) and the prospective design. However, additional studies are needed to conclusively demonstrate a link between antibiotic use in early life and food allergy risk.

Only a few studies have explored the relationship between food allergies and antibiotic use. The committee concludes that evidence from observational studies suggesting a link between antibiotic use in early life and food allergies is limited. Additional studies with information on the type and dose of antibiotic, the timing of exposure along the perinatal continuum, and whether the infant is repeatedly exposed are needed to conclusively demonstrate a link with food allergies.

Animal Exposure

As noted above, the premise of the "Hygiene" and "Old Friends" hypotheses is based on the concept that the lack of early childhood exposure to infectious agents, symbiotic microorganisms, and/or parasites increases susceptibility to allergic diseases and asthma by suppressing the natural development of the immune system (Strachan, 1989).

The Marrs review reported on four studies investigating associations between farm and animal exposure and food allergy (Marrs et al., 2013). In their review, only the HealthNuts Study supported the microbial hypothesis. The study reported data on risk of pets and siblings for the develop-

ment of challenge-proven egg allergy (Koplin et al., 2012a). It also assessed the role of these factors on any food allergy using latent class analysis, a sophisticated analytical epidemiological method (Peters et al., 2015). Marrs et al. also reported findings from the European Protection against Allergy Study in Rural Environments (PASTURE), which described a cohort of families living in proximity to farm animals in rural settings (Marrs et al., 2013). This study showed significantly less food sensitization in the cord blood of mothers who consumed raw cow milk (versus boiled milk) in the perinatal period. However, the authors applied a lower cutoff for sIgE concentration than is conventionally used (>0.2 versus 0.35 IU/ml), which may have overestimated the incidence of food sensitization (Ege et al., 2008).

Since 2012, several prospective cohort studies have investigated whether exposure to farm animals (Depner et al., 2013; Pele et al., 2013) or pets (Goldberg al., 2013; Grimshaw et al., 2014; Martin et al., 2015; Peters et al., 2015; Stelmach et al., 2014) influenced the risk of food allergy or food sensitization. Depner et al. performed an additional analysis of data from 686 children in the rural European PASTURE cohort (Depner et al., 2013). Again using sIgE as their diagnostic criterion for food sensitization, they explored the more traditionally used sIgE cutoff of 0.35 IU/ml compared to 0.2 IU/ml in their previous study by Ege et al. (2008). They found that allergen-specific IgE levels rarely exceeded 0.35 IU/mL (<3% of all children) at age 1 year and the 95th percentiles at 1 year were consistently less than 0.7 IU/mL (RAST class 2) for any IgE. The only exception was cat (1.3 IU/mL) (Depner et al., 2013). They also found that early life exposure to farm animals, such as sheep, goats, and rabbits, did not confer protection against food allergen sensitization. However, exposure to farming increased (P=0.0015) the risk of food allergen sensitization (aOR: 2.11; 95% CI: 1.33-3.34). A total of 793 (378 farm and 415 nonfarm) children were included in the analyses. Pele et al. also reported no effect of farm animal contact on food allergy incidence in more than 1,400 children participating in the PELAGIE mother–child cohort. However, mold or dampness in the home increased (P≤0.001) the incidence of food allergy (23.9% versus 8.8%, yes versus no) in this cohort, as measured by parent report (Pele et al., 2013).

All other prospective cohort studies published since 2012 investigated exposure to pets. Two studies with a total of 350 children reported no association between pets in the home (Israel) and food sensitization (measured by specific IgE to cow milk) (Goldberg et al., 2013) nor an association of pet ownership (United Kingdom) with food allergy risk (measured by DBPCOFC or convincing history of anaphylaxis) (Grimshaw et al., 2014). In contrast, Stelmach et al. reported an increased risk of food allergy based on diagnosis by a doctor following international guidelines (aOR: 1.48; 95% CI: 1.02-2.16) associated with pets in the home during pregnancy in

a cohort of 501 children from the Polish Mother and Child Cohort Study (REPRO_PL cohort) (Stelmach et al., 2014).

Two studies from the HealthNuts cohort, a prospective, population-based cohort of 5,276 infants age 12 months in Melbourne, Australia, investigated whether direct exposure to pets (Koplin et al., 2012a; Peters et al., 2015) or the co-incidence of eczema (Martin et al., 2015) moderated the effect of pets on food allergy risk. Koplin et al. examined the relationship between environmental and demographic factors and egg allergy, the most common food allergy in infants and young children (Koplin et al., 2012a). Using SPT to egg white and oral food challenge at 12 months revealed that children with a pet dog at home (dog ownership ascertained by questionnaire) were less likely to develop egg allergy than those without a pet dog at home (aOR: 0.72; 95% CI: 0.52-0.99). Peters et al. observed that, compared to not having a dog in the home, having a dog significantly reduced the risk of multiple food allergies (including peanut) by 60 percent (aOR: 0.4; 95% CI: 0.21-0.73), whereas having a dog that was kept outside only (versus no dog) provided no protection. In this latter scenario, a significantly increased risk in egg allergy was actually observed (aOR: 1.56; 95% CI: 1.1-2.21) (Peters et al., 2015). Within the same cohort, Martin et al. compared the effect of dog or cat exposure on infants with (N=2,795) or without (N=1,903) eczema (Martin et al., 2015). Having a dog reduced the risk of food allergy in infants with eczema (aOR: 0.7; 95% CI: 0.5-0.9), but not in infants without eczema. A similar effect on food allergies was observed for infants with (aOR: 0.6; 95% CI: 0.4-0.9) or without eczema in homes with cats (Martin et al., 2015).

Results from studies exploring the relationship between animal exposures and food allergies are inconsistent. The few observational studies related to living on a farm found that exposure to farm animals offers no protection against food allergies. Also, from observational studies, the committee concludes that evidence is limited regarding the potential for a close interaction with a pet being more protective against a food allergy than pet ownership in general or having a pet who is restricted to outside the home. Further studies should be conducted on the nature of the association between exposure to farm animals or pet ownership and food allergies.

Allergen Avoidance Hypothesis

As mentioned in the introduction of this chapter, in considering the risk determinants for developing food allergies, the committee focused on the prenatal and early childhood developmental periods. In that vein, this section focuses on allergen exposure beginning at conception. The allergen avoidance hypothesis was predicated on the basis of the concept that

avoiding common food allergens early in life when the immune system is developing would prevent the onset of food allergies.

Exposure to Antigen Through Maternal Diet During Pregnancy or Lactation

Maternal diet during pregnancy and lactation has been of great interest in understanding the etiology of food allergies in offspring. The fetal programming hypothesis supports the idea that the maternal diet has long-term influence on children's health (Barker, 1990; Langley-Evans, 1997). Its application to food allergies would suggest that consuming specific allergenic foods during this critical period might be associated with the development of the immune system in utero that may later manifest itself as food allergies over the life course, given specific childhood exposures. Results from two prospective cohort studies (Bunyavanich et al., 2014; Frazier et al., 2014) (total N=9,482 mother–child pairs) show that a higher consumption of allergenic foods before or during pregnancy (e.g., peanut), as measured by a food frequency questionnaire, was associated with a reduced risk of having a child with food allergies. This finding supports the fetal programming hypothesis. The HealthNuts Study also assessed the role of allergen avoidance in pregnancy and lactation and the risk of challenge-proven egg allergy and found no association (Koplin et al., 2010). Another recent prospective cohort study (Pele et al., 2013) reported an association between maternal pre-pregnancy consumption of shellfish and food allergy (1.62; 95% CI: 1.11-2.37). However, this study assessed food allergy by parental report. Randomized studies on this subject have involved the elimination of certain allergenic foods as opposed to increasing their consumption among primarily high-risk families. Kramer and Kakuma conducted a high-quality systematic review that included three RCTs of foods avoided during pregnancy and/or lactation and the outcomes of egg and milk sensitization (but not food allergy itself) among women at high risk of having an atopic offspring (Kramer and Kakuma, 2012). In two of the RCTs (Falth-Mangnusson and Kjellman, 1987; Lilja et al., 1988) (total N=334), women either avoided or decreased their intake of cow milk and eggs beginning in the third trimester of pregnancy and this was associated with a nonsignificant reduction of egg sensitization in their infants at 6 months, but not at 18 months. Sensitization for cow milk allergy was not reduced at either time point. The remaining RCT (Appelt et al., 2004) (total N=497) had women totally avoid peanuts, nuts, and fish as well as decrease their intake of cow milk and eggs beginning in the third trimester through 1 year postpartum. This study found no significant associations with milk or peanut sensitization in offspring at age 1, 2, or 7 years. However, for egg sensitization, an increased risk was seen at age 2 years only (1.91; 95% CI: