Journal of Applied Biomedicine 15 (2017) 161–168

Contents lists available at ScienceDirect

Journal of Applied Biomedicine

journal homepage: www.elsevier.com/locate/jab

Review Article

Hits and defeats of genome-wide association studies of atopy and asthma

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ARTICLE INFO

Article history:
Received 16 November 2016
Received in revised form 7 March 2017
Accepted 7 March 2017
Available online 24 March 2017

Keywords:
GWAS
Asthma
Atopy
Gene-environment
Epigenetics
Farming

ABSTRACT

Atopy and asthma are complex conditions, recognised as outcomes in which both genes and environment play crucial role. Large number of disease associated loci have been identified within GWAS approach over last years and the knowledge of pathobiology of asthma and allergy has widened substantially. However still the results achieved are difficult to interpret. Most markers have no clear function on and expound small portion of heritability. The "missed heritability" could be hidden in the gene-by environment interactions. The most know environmental factor which interacts with asthma and atopy is farming. The common link between genes and environment could be epigenetics.

Introduction

Asthma and allergic atopic conditions are complex traits, recognised as outcomes in which both genes and environment play an important role. The family studies with segregation analysis and twin studies brought the evidences of heritability, but the increase in the prevalence could not be explained by genetic drift. Thus the importance of an environmental component is commonly in focus. Asthma is believed to be also triggered by developmental factors early in life, which currently are poorly recognised. An example of that relationship is correlation of asthma at 10 years with reduced lung function at birth (Håland et al., 2006). Similarly genes implicated in foetal lung development have been showed to influence asthma susceptibility and treatment response (Sharma et al., 2015).

Different environmental exposures have been taken into account as impacting allergy – urban vs. rural living, farm vs. non-farm, change of diet, increased air pollution, reduced infections, parasite exposure and increased use of antibiotics. They can be considered as components of well-known hygiene hypothesis. The umbrella term "hygiene" or "the lack of hygiene" is currently replaced by biodiversity. Biodiversity hypothesis implicates that reduced biodiversity has negative consequences as a reduced immune tolerance to "harmless" allergens. Immunomodulatory role of saprophytic bacteria has been recognised as a main benefit of biodiversity. Last findings in that subject emphasise the role of farming in the richness of microbiome composition and diversity (Birzele et al., 2017; Depner et al., 2017).

Studying genes – genome-wide association study GWAS

Essential number of genetic studies has been conducted in order to find out the potential genetic risk associated with allergic conditions. In GWAS, which is relatively new approach, genome scan is used in the hypothesis independent manner, which allows to identify multiply candidate genes for complex diseases. In the statistical analysis a test for the association between common genetic variants spread throughout the whole genome is performed. Contrary, well known, candidate gene strategy is hypothesis driven – based on the current knowledge in the area. The problems associated with that kind of research are frequent discrepancies between studies, some caused by population stratification or small sample size. Furthermore, this kind of analysis doesn't allow discovering novel genes or molecular pathways.

Single nucleotide polymorphism is one of the most common type of variation within human genome, which occurs in the population with frequency of at least 1%. SNP appears with an average of 1 per 300 bases, so it is estimated that approximately

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http://dx.doi.org/10.1016/j.jab.2017.03.002
1214-021X/

Nomenclature

ADA1	Adenosine deaminase 1
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif 9
AP5B1	Adaptor related protein complex 5 beta 1 subunit
ASB3	Ankyrin repeat and SOCS box containing 3
BCAP	B-Cell adapter for phosphoinositide 3-Kinase
C11orf30	EMSY, BRCA2 interacting transcriptional repressor
CARD4	Caspase recruitment domain family member 4
CDH17	Cadherin 17
CEP68	Centrosomal protein 68
CLEC16A	C-Type lectin domain family 16 member a
COL18A1	Collagen type XVIII alpha 1
COL29A1	Collagen type XXIX alpha 1
CTNNA3	Catenin alpha 3
DAD1	Defender against cell death 1
DENND1B	DENN domain containing 1B
DEXI	Dexi homolog
EFHC1	EF-Hand domain containing 1
FCER1A	High affinity immunoglobulin epsilon receptor alpha-subunit
FLG	Filaggrin
FNDC3A	Fibronectin type III domain containing 3A
FOXA1	Forkhead box A1
FOXB1	Forkhead box B1
GAB1	GRB2 associated binding protein 1
GATA2	GATA binding protein 2
GLCCI1	Glucocorticoid induced 1
GSD1A	Glucose-6-phosphatase catalytic subunit
GSDMA	Gasdermin A
GSDMB	Gasdermin B
GWAS	Genome-wide association study
HERC2	HECT and RLD domain containing E3 ubiquitin protein ligase 2
HIF-1α	Hypoxia inducible factor 1 alpha subunit
HLA-DPB1	Major histocompatibility complex, class II, DP beta1
HLA-DQ	Major histocompatibility complex, class II, DQ
IKZF2	IKAROS family zinc finger 2
IKZF3	IKAROS family zinc finger 3
IL13	Interleukin 13
IL18R1	Interleukin 18 receptor 1
IL1RL1	IL-33 receptor
IL2RA	Interleukin 2 receptor subunit alpha
IL2RB	Interleukin 2 receptor subunit beta
IL4	Interleukin 4
IL5	Interleukin 5
IL33	Interleukin 33
KIF3A	Kinesin family member 3A
LPP	LIM domain containing preferred translocation partner in lipoma
LRRC32	Leucine rich repeat containing 32
LRRC32	Leucine rich repeat containing 32
MAP3K5	Mitogen-activated protein kinase 5
MICA	MHC class I polypeptide-related sequence A
MLLT3	MLLT3, super elongation complex subunit
MRPL4	Mitochondrial ribosomal protein L4
MYB	MYB proto-oncogene, transcription factor
MYC	V-Myc avian myelocytomatosis viral oncogene homolog
NFATC2	Nuclear factor of activated T-Cells 2
NF-KB	Nuclear factor kappa B
NOD1	Nucleotide binding oligomerization domain containing 1
ODZ3	Alias TENM3 teneurin transmembrane protein 3
ORMDL3	ORMDL sphingolipid biosynthesis regulator 3
OVOL1	Ovo like zinc finger 1
OXA1L	Oxidase (Cytochrome C) assembly 1-Like
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase catalytic subunit alpha
PLCL1	Phospholipase C like 1
PTGER4	Prostaglandin E receptor 4
PYHIN1	Pyrin and HIN domain family member 1
RAD50	RAD50 double strand break repair protein
RORA	RAR related orphan receptor A
SgK493	Sugen kinase 493 (Protein kinase domain containing, cytoplasmic PKDCC)
SH2B3	SH2B adaptor protein 3
SLC25A46	Solute carrier family 25 member 46
SLC6A15	Solute carrier family 6 member 15
SMAD3	SMAD family member 3
SNP	Single nucleotide polymorphism
STAT6	Signal transducer and activator of transcription 6
TBCD	Tubulin folding cofactor D
TMEM232	Transmembrane protein 232
TMTC2	Transmembrane and tetratricopeptide repeat containing 2
TNIP1	TNFAIP3 interacting protein 1
TNS1	Tensin 1
TSLP	Thymic stromal lymphopoietin
TTC6	Tetratricopeptide repeat domain 6
USP38	Ubiquitin specific peptidase 38
WDR36	WD repeat domain 36
ZBTB10	Zinc finger and BTB domain containing 10

there are 10 million variants per single genome with 3 billion nucleotides. Some of SNPs are localised around the genes, influencing the variation in the amount or the function of a protein (International HapMap Consortium, 2003). According to current dbSNP database (NIH) there are 154.2 million SNPs in total, with the number increasing every year (dbSNP, 2017). Due to haplotype structure of the genome only 1 million SNPs could capture 90% of the population variation (Lander, 2011). Conferring to common disease-common variant hypothesis SNPs considered useful for genome wide association studies in most queries had a minor allele frequency of at least 5%. The SNPS with MAF $<1\%$ are considered rare, and 1–4% low-frequency and usually are excluded from the analysis (Ober, 2016). The GWAS microarrays are developed with a reference to linkage disequilibrium (LD). They are now able to overlay up to 5 million SNPs. This approach localises the susceptibility locus with size 10–500 kb. A genome wide threshold level is used for statistic 5×10^{-8} if a test of independence is exerted for 1 million SNP with $p < 0.05$, because of multiple comparisons. Even with this cautious, any finding revealed, should be replicated in additional population, due to the possibility of false positive results, as it is true for all association studies (Tamari et al., 2013).

In the last few years, a number of GWAS studies for atopy and asthma have been conducted which allows the ability to detect multiple susceptible loci (Portelli et al., 2015). When for asthma in the 2015 there were five studies, and multiple in 2014, for atopy the last findings (three studies) are from publication from 2011 (GWAS catalogue, 2017). Also for atopy there are three large meta-analysis using existing GWAS data.

GWAS in atopic phenotypes

One of first GWAS-atopy study, conducted in 1530 subjects, looked for a relationship between genetic markers and total serum IgE in connection with allergic sensitisation (Weidinger et al., 2008). Functional variants in FCER1A gene on chromosome 1q23 revealed strong association. The study also confirmed the role of STAT6 and locus on chromosome 5q31 with the cluster of IL4 and IL13, however the exact casual polymorphism has not been found. Wan et al. (2011) conducted GWAS of an elevated total IgE in the population of the UK. The SNP rs6561505 in intron 2 of FNDC3A gene encoding fibronectin III has been revealed as significant in this respect. However, the replication study failed to confirm these findings. Andiappan et al. (2011) suggested that MRPL4 and BCAP, key components of the HIF-1α and PI3 K/Akt signalling pathways respectively, were two novel candidate genes for atopy and allergic rhinitis. Miller et al. (2011) reported two association for vaccine related wheezing in infancy but none showed genome wide significance. Castro-Giner et al. (2009) revealed that SgK493 (SGK493) was found to be associated with atopy, also to lesser extent mitogen-activated protein kinase 5 (MAP3K5), collagen type XVIII alpha 1 (COL18A1) and collagen type XXIX alpha 1 (COL29A1). Gudbjartsson et al. (2009), within the GWAS of eosinophilic count – which is considered as atopy associated phenotype, revealed 7 associations, between them gene encoding IL-33 receptor (IL1RL1) and Th2 promoting cytokine IL33, together with gene regulating hematopoietic progenitor cells (IKZF2).

Ramasamy et al. (2011) performed the meta-analysis using existing GWAS for self-reported allergic rhinitis and grass sensitization phenotypes. 2.2 million genotyped or imputed SNP were analysed in 4 large European cohorts. Polymorphism rs7775228 in HLA region which *cis*-regulates HLA-DRB4, variants in the locus near C11orf30 and LRRC32 (indicated previously as associated with atopic dermatitis) and rs17513503 located near TMEM232 and SLC25A46 with proximity to TSLP, have been shown to be relevant with genome wide significance. Additional analysis with the gene-candidate approach revealed TSLP, TLR6 and NOD1/CARD4 to be important. Within this analysis no gene-environment interaction was showed even though the epidemiological data suggested the birth order as a modulator of genetic effect.

Bønnelykke et al. (2013) conducted another meta-analysis using data from 16 studies from EAGLE and AAGC Consortia revealing 10 loci containing risk-associated variants which accounted for 25% of allergic rhinitis or atopic sensitization. The study was enriched with additional QTLS analysis, replication and also an association study in separate populations. Loci in or near TLR6, C11orf30, STAT6, SLC25A46, HLA-DQB1, IL1RL1, LPP, MYC, IL-2, HLA-B have been reported there, as significant.

The third meta-analysis was performed by Hinds et al. (2013). The data from 23andMe cohorts and ALSPAC cohort was used for analysis. In the group of 53862 individuals with self-reported cat, dust mites or pollen allergy 16 loci showed shared susceptibility pattern and one – in HLA region – allergen specific (cat), 8 of them were also associated with asthma in previous studies. Top loci were – 4p14 near TLR1, 6, 10, 6p21.33 near HLA-C and MICA, 5p13.1 near PTGER4, 2q33.1 in PLCL1, 3q28 in LPP, 20q13.2 in NFATC2, 4q.27 in ADAD1, 14q21 near FOXA1 and TTC6. What's more some of these loci have been described previously to be associated with autoimmune disease. Summarised in Table 1.

GWAS in asthma

Several GWAS of asthma have been performed. The most known finding from GWAS studies in the European population are the results from Moffatt et al. (2007) and GABRIEL Consortium survey (Moffatt et al., 2010) with 10 365 cases and 16 110 controls is OMRDL3/GSDMA/GSDMB on chromosome 17q12-21.1–which encodes proteins with an unknown function, but associated with childhood asthma, genes IL1RL1/IL18R1, HLA-DQ, IL33, SMAD3and IL2RB, which cover functions of innate immunity cells, epithelial barrier, IL-1 family signalling, regulatory T cells and vitamin D pathways. Also RORA and IL13 were suggestive. Another GWAS analysis with subsample of GABRIEL population was performed by Melén et al. (2013) for asthma and obesity indicating DENND1B as a plausible gene DENND1B gene on chromosome 1q31 was initially described as associated with asthma by Sleiman et al. (2010) in US diverse population. Li et al. (2010) performed GWAS on difficult to treat asthma, finding out loci RAD50, IL13 and HLA DR DQ positively associated. DeWan et al. (2010) indicted PDE11A as relevant gene associated with childhood asthma. Ferreira et al. (2011) revealed locus of IL6R and on chromosome region 11q31–without a known candidate within this location. Hirota et al. (2011) performed GWAS in Japan, showing genes USP38 and GAB1 to be important. Also Torgerson et al. (2011) conducted meta-analysis of GWAS in multi-ethnic populations and revealed locus PYHIN1 with a strong impact in population with African ancestry. Tantisira et al. (2012) revealed loci GLCCI1 for GKS responsive asthma. Wan et al. (2012) suggested variants within IL18R1 as risk factors for adult asthma. In another study Li et al. (2012) revealed new candidate – TNIP1 as associated with asthma and autoimmune disorders, with opposite effect. Some other polymorphisms – within IL-13, HLD-DRA and GSDMB showed the same pattern. rs1422673 in TNIP1 was also replicated in GABRIEL and EVE populations. Park et al. (2014) indicted ALCC gene as related to GKS response in asthmatic patients. Wang et al. (2015a, 2015b) described 5 loci associated with dose-dependent response to inhaled steroids in asthma, with two showing increased significance. Similarly Dahlin et al. (2015) showed association of several loci with the response to montelukast, with one – rs6475448 MMLT3, showing genome wide significance. McGeachie et al. (2015) conducted GWAS for the exacerbation of asthma and found out that locus in CTNNA3 region reached genome-wide significance. Park et al. (2015) revealed genetic risk factors for decreased bone mineral accretion in children with asthma receiving multiple oral corticosteroid courses. Israel et al. (2015) showed a novel genome wide significant locus on chromosome 2, near ASB3. Costa et al. (2015) presented some association for regions 14q11, flanking genes DAD1 and OXA1L, and 15q12 within the region of FOXB1 gene and childhood asthma in Latin American population, however failing in

Table 1
Susceptibility loci of atopy identified by GWAS.

atopy	
FCER1A, STAT6, 5q31 (IL4 and IL13)	Weidinger et al. (2008)
SgK493 (SGK493), MAP3K5, COL18A1,COL29A1	Castro-Giner et al. (2009)
IL1RL1, IKZF2, GATA2, IL5, SH2B3, WDR36, MYB, IL33	Gudbjartsson et al. (2009)
HLA-DRB4, C11orf30, TMEM232/SLC25A46/TSLP	Ramasamy et al. (2011)
TLR6, C11orf30, STAT6, SLC25A46, HLA DQB1, IL1RL1, LPP, MYC, IL-2, HLA-B	Bønnelykke et al. (2013)
TLR1, TLR6, TLR10, HLA-C/MICA, PTGER4, PLCL1, LPP, NFATC2, ADAD1, FOXA1/TTC6	Hinds et al. (2013)

replication of original results. The last known study, focused on Spanish population, revealed in 2015 ADAMTS9 gene as plausible candidate for asthma association (Barreto-Luis et al., 2016).

Considering asthma genetics it's crucial to well define asthma phenotypes. Not only the adult/childhood origin and phenotypes associated with response to treatment are relevant but also endotypes such as IgE dependent-atopic asthma and non-atopic asthma, aspirin exacerbated asthma, occupational asthma, exercise induced asthma, and phenotypes related to menstruation and obesity. Several finding were published for this phenotypes. GWAS study for TDI-induced asthma by Kim et al., (2009) revealed CTNNA3 gene as significant. Yucesoy et al. (2015) found association for isocyanate-induced occupational asthma – genes HERC2, ODZ3/CDH17. Aspirin exacerbated asthma had been found to be associated with CEP68 (Kim et al., 2010) and HLA-DPB1 (Park et al., 2013). Summarised in Table 2.

Comorbidity

The studies of heritability suggest that genes predisposed to asthma overlap those predisposed to atopy, however the true overlapping in GWAS studies is strikingly low. In the GABRIEL Consortium study, loci strongly associated with IgE were not associated with asthma, except for IL-13 and HLA (Moffatt et al., 2010). In 2011 two studies revelead gene C11orf30/LRRC32 on chromosome 11 seemed to be promising candidate for asthma-eczema-allergic rhinitis phenotype (Marenholz et al., 2011; Ramasamy et al., 2011). GWAS performed in Korean asthmatic population haven't revealed any specific SNP for association with total IgE and dust mites specific IgE however loci within genes CRIM1, ZNF71, TLN1, SYNPO2 have been suggested to be promising (Kim et al., 2013). More recent study revealed 11 loci associated with having asthma with hay fever, among them ZBTB10, CLEC16A, locus near DEXI and LRRC32, loci near GSD1A and TSLP and locus near IL2RA and in TNS1 (Ferreira et al., 2014). Very recent meta-analysis concerning atopic march defined as eczema followed by asthma revealed 5 loci, previously reported to be associated with atopic phenotypes – FLG (1q21.3), IL4/KIF3A (5q31.1), AP5B1/OVOL1 (11q13.1), C11orf30/LRRC32 (11q13.5) and IKZF3 (17q21) and two novel – EFHC1 on chromosome 6p12.3 and SNP on chromosome 12q21.3 between genes TMTC2 and SLC6A15. What is interesting genes triggering eczema seemed to be predominate for affecting the atopic march witch highlight the role of skin barrier as the interaction site for gene-environment foreplay (Marenholz et al., 2015). Summarised in Table 3.

GWAS results – questionable?

As it can be seen within 10 years large number of diseases associated loci has been identified with the use of GWAS approach and the knowledge of pathobiology of asthma and allergy have widened substantially. Still the results achieved are difficult to interpret. Most markers have no clear function on aetiology and explain a small proportion of heritability. The explanation of that is possibly our limited understanding of genome function especially in non-coding regions, in which a considerable number of disease-associated loci have been discovered. The "missing heritability" could lay also in the genomic aberrations such as insertion and deletion, low LD, epigenetic variance, gene–gene interactions, phantom heritability and as well, the most interesting with gene-by environment interactions (Holloway 2014; Wang et al., 2015a, 2015b). Rare variants are considered as the alternative explanation of "missing heritability". Both the 1000 Genomes Project Consortium et al. (2012) and National Heart, Lung and Blood Institute [NHLBI]–funded Exome Sequencing (Exome Variant Server, 2017) (includes variants relevant to the alternation of specific protein) have given the deeper pattern of rare variants. It was discovered that large proportion of single nucleotide variants across different population are rare variants with MIF $< 1\%$. Some of them, which were not included in the GWAS platforms, can be identified only through the next-generation sequencing (NGS – simultaneous sequencing of thousands to millions of short nucleic acids segments in massive fashion). Human exome contains approximately 30 million base pairs, which is 1% of human genome. Recently developed exome chip includes 240 000 protein-altering variants ($>$1:1000), also containing missense, splice-site, nonsense variation and enable to capture 95% of exome variation. Specific computational methods can be used for combing the traditional SNP genotyping with exome chip analysis, thus increasing the imputation accuracy for rare variants (Kim et al., 2015). Anyway, till

Table 2
Susceptibility loci of asthma identified by GWAS.

asthma	
OMDRL3/GSDMBA/GSDMB, IL1RL1/IL18R1, HLA-DQ, SMAD3, IL2RB	Moffatt et al. (2007, 2010)
DENND1B	Melén et al. (2013), Sleiman et al. (2010)
RAD50, IL13, HLA DR DQ	Li et al. (2010)
PDE11A	DeWan et al. (2010)
IL6R, region 11q31/LRRC32	Ferreira et al. (2011)
USP38, GAB1	Hirota et al. (2011)
PYHIN1	Torgerson et al. (2011)
GLCCI1	Tantisira et al. (2012)
IL18R1	Wan et al. (2012)
TNIP1, IL-13, HLD-DRA, GSDMB	Li et al. (2012)
chr6 rs6924808, chr11 rs1353649	Wang et al. (2015a, 2015b)
MMLT3	Dahlin et al. (2015)
CTNNA3	McGeachie et al. (2015)
TBCD	Park et al. (2015)
ASB3	Israel et al. (2015)
DAD1/OXA1L, FOXB1	Costa et al. (2015)
ADAMTS9	Barreto-Luis et al. (2016)
Asthma sub-phenotypes	
CTNNA3	Kim et al. (2009)
CEP68	Kim et al. (2010)
HLA-DPB1	Park et al. (2013)
HERC2, ODZ3/CDH17	Yucesoy et al. (2015)

Table 3
Susceptibility loci of comorbid asthma-atopy phenotype identified by GWAS.

Comorbidity asthma-atopy	
IL-13, HLA	Moffatt et al. (2010)
C11orf30/LRRC32	Marenholz et al. (2011) Ramasamy et al. (2011)
ZBTB10, CLEC16A, DEXI/LRRC32, GSD1A/TSLP, IL2RA, TNS1	Ferreira et al. (2014)
FLG, IL4/KIF3A, AP5B1/OVOL1, C11orf30/LRRC32, IKZF3, EFHC1, TMTC2/SLC6A15	Marenholz et al. (2015)

now even focusing on the rare variants hasn't explained much in the picture of asthma susceptibility (Ober, 2016).

Studying environment

The most known environmental protective factor for asthma and atopy is farming with biodiversity associated with that kind style of life. The protective exposure components associated with farming are complex and heterogeneous. The diversity of microbial components is important and the milieu of endotoxins, peptidoglycans, gram-positive bacteria and fungi play the key role. In many epidemiological studies, the protective role of farm living was confirmed. However, while for IgE sensitisation and atopy the results are excellent, they are inconsistent for asthma. The problem is that different studies did not measure the farm effect equally – mainly because of cultural difference in the "style" of farming. Thus, the exposure estimates are different (Genuneit, 2012).

Moreover, many upper and lower respiratory diseases are associated with chronic farming exposure. It affects mainly adults, probably because of the duration of exposure. Sinusitis, rhinitis, asthma and hypersensitivity pneumonitis are the respiratory outcomes associated with negative impact of farming. As well, subjects with diagnosed allergic condition could experience a worsening of symptoms due to farming exposure. Also, it seems to be that an initial exposure to farm animals as an adult could harbour risk of allergic sensitisation. In the USA, it is observed that farming is not protective from asthma and there is even a higher risk of asthma associated phenotypes with swine exposure with prevalence of asthma up to 44%, and for swine breeding with antibiotic use for feeding up to 56% of exposed. Chronic bronchitis is diagnosed up to 32% of farmers dealing with animal breeding. Animal farming seems to be at a higher risk to developed airway disease than crop farming. The risk seems to be higher also in large scale farms, closed and related to concentrated animal-feeding operations with high exposure to organic dust. High exposure to endotoxin was revealed to be associated with non-atopic wheeze also in European countries – Austria, Germany and Switzerland (Poole, 2012).

The main question raised here is if the farm is protecting, and what is the interaction between atopy and asthma in childhood with the implication of farm exposure. GABRIEL study was one of largest epidemiological studies in central Europe regarding the influence of both farming environment and genetic heritability.

The GABRIEL phase I and phase II took place in five rural regions, two in Germany, and one each in Switzerland, Austria and western Poland – Lower Silesia. A protective role of farm environment has been confirmed there. Farming in Lower Silesia differed from that in "Alpine centres" – for example cattle farming was dominant in the Alpine region, whereas in Poland 18% of village farms kept cows and 34% pigs with predominant pattern of small number of animals, however similarly to Alpine's, Polish children in rural villages had a lower prevalence of childhood asthma and hay fever than children from towns. Farm children had also a reduced risk of atopy measured by IgE and skin prick test. Early-life contact with grain was inversely related to the risk of atopy. An 'exposure-response' effect was observed – the number of various farm exposure episodes was revealed to be increasingly protective against atopy (MacNeill et al., 2013).

Ege et al. (2011) performed very specific kind of analysis, focused on gene-environment interaction within GABRIEL population (GWIS – genome wide interaction study for gene-environment interaction) using an array with 500 000 SNPs (Ilumina human 610) and 1708 subjects from "Alpine GABRIEL centres" which were assessed for interaction with several farm related exposure episodes, i.e.: living on a family-run farm, mother who grew up on a farm, regular consumption of raw farm milk, regular contact with cows, regular contact with straw, regular contact with hay, and coincidence of cow and straw exposure. Neither 7 SNPs emerged as genome-wide significant in meta-analysis of childhood asthma (Moffatt et al., 2010) nor those published previously for interaction with an environment showed significant association.

Newest study in this subject performed by Stein et al. (2016) compared the unique population Amish of Indiana and Hutterites of South Dakota. Both these population share common European ancestry but their style of life differs substantially in the terms of mode of farming. While Hutterites provide highly industrialized farms, Amish prefer more traditional, usually single family-led farms. These differences seem to be responsible for the gap in the prevalence of both asthma and allergic sensitization in these two groups, indicating Hutterites to have higher prevalence. In Stein's study, it was revealed that this different pattern of farm exposure relates to different immune profile of specific blood cells, as well as gene-expression pattern. The genes which were differentially expressed were those affecting innate immunity: TNFAIP3 encoding A20 which modulates the inflammation pathways activated by NF-kB, IRF7 representing innate response to viruses and TRIM8, which is the regulator of TNF alpha and IL1B-dependent induction of NF-kB.

Environment vs. genes – epigenetics?

Studying genes which interact with the environment is nowadays in the focus of research. The common link between genes and environment could be epigenetics.

Epigenetic studies search for heritable changes in gene expression or cellular phenotypes that do not involve changes in the DNA. Two main epigenetic mechanisms are DNA methylation and post-translational histone modification. DNA methylation is an ancient adaptation which allows to distinguish own DNA from others such as viruses. It is the proof of plastic response to environmental exposure and controls gene activity. DNA methylation is affected by both genetic variants and environmental exposure. Genome alone explains 25% of variability in methylation, while 75% is explained by environment. It means that DNA methylation could be allele specific – different SNPs abolish or activate methylation. As an example of environmental impact, placental methylation level of CD14 gene was shown to correlate with the expression level of CD14 in women living on a farm, which correlates to specific exposure. In the PASTEURE study, it was

revealed that farm environment is associated with hypomethylation in the ORMDL1 and STAT6 genes, and hypermethylation in the RAD50 and IL13 genes (Devries and Vercelli, 2013; Kabesch, 2014; Michel et al., 2013). EWAS studies were conducted for DNA methylation with other environmental exposures – such as prenatal tobacco smoke exposure (Rzehak et al., 2016) and PAH exposure (Tang et al., 2012). The information elucidated from these researches are sometimes regarded as more valuable than for standard diseases oriented GWAS. For example, GWAS for total IgE have indicted association for genes STAT6, FCER1A IL4/RAD50 MHC – but they account for only 1–2% variability of serum IgE, while GWAS for methylation and total IgE revealed low methylation at 36 loci, and the 3 top loci account for 13% variability of total IgE. Genes annotated to these loci encoded known eosinophilic products (Liang et al., 2015). A specific methylation pattern has been found for genes already known to be differently expressed in asthmatics and non-asthmatics. For example, IL4 expression has been related to an upstream epigenetic variation in DNA methylation in T cells (Harb and Renz, 2015). What seems to be problematic in methylation studies, is the changing pattern of methylation in the disease course and under the treatment (Hong and Wang, 2014). This is due to a changing over time and under different stimuli constituting an epigenetic drift over the life course. But what is the problem could also be a solution – if the gene expression level is changeable by different environment stimuli we can also change and cure the disease. Nevertheless, epigenetic study in asthma and atopy are still in a preliminary stage.

Conclusion

GWAS is excellent tool for genetic research in term of atopy and asthma. However, it seems to be necessary to enrich the analysis with some functional studies, not only focused on gene function, as are genome wide expression analysis, but as well provided with "put in" the environmental exposure element through the assessment of the genetic risk of specific gene pattern.

Conflict of interests

I declare, that I have received honorarium payment for the lecture from G-Phrama Consulting, adsministrator of the Allergy and Pulmonology Conference.

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