Torabian et al., 2018. Journal of Genes & Cells, 4(2): p, 67-75
doi:10.15562/gnc.65

REVIEW

Genetics and Epigenetics of Celiac disease: Heads or Tails?

Pedram Torabian[1], Mohamad Dehestani[2], Azra Izanloo[1], Narges Jafarzadeh[1], Azita Ganji[3], Kamran Ghaffarzadegan[1]*

1- *Razavi Cancer Research Center, Razavi Hospital, Imam Reza International University, Mashhad, Iran*
2- *Medical Genetics Research Center (MGRC), School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
3- *Department of Internal Medicine, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran*
4- **Corresponding author: Kamran Ghaffarzadegan, Razavi Cancer Research Center, Razavi Hospital, Imam Reza International University, Mashhad, Iran*

ABSTRACT

Celiac is a serious gluten sensitive enteropathy which is caused by autoimmune reaction based on genetics and epigenetics factors. About 3 million Americans suffer from this disorder. The symptoms occur when gluten is consumed and thereafter, autoimmune responses manifest. This disorder is first described in a child but it may happen at any age especially after impacts of triggers. Many genetics and epigenetics are involved in this disease including HLA genes DQ and other like CTLA4, IL2, IL21, MYO9B, etc. Considering the epigenetics factors, histon modifications and also miRNAs activities inside the body is verified and the affection is investigated. To unravel new treatments for celiac disease, new approaches for instance next generation sequencing in affected people is desired and look for modern and also alternative treatments are needed.

Keywords: Celiac disease, gluten, genetics, epigenetics, next generation sequencing

Celiac disease (CD) is regarded as a chronic small-intestine disorder characterized by mucosal injury. This disorder is caused by the body's immune system countering to proteins in wheat, barley and rye in genetically susceptible individuals. The main reason of this nutrient malabsorption is triggered by the dietary ingestion of proline and glutamine rich proteins, broadly termed "gluten". Henceforward, the body is not able to absorb momentous vitamins, minerals and calories. Manifesting a spectrum of digestive symptoms, the affected people are not diagnosed, though. The approximate prevalence is 1% of the population of the United States and Europe (1) and is increasing in incidence possibly as a result of enhancement of diagnostic methods. Diagnosis is based on detection of IgA antibody specific tissue transglutaminase and should be confirmed by biopsy of the mucosa of the small intestine to set up a guaranteed diagnosis (2).

* Correspondence:
kghafar46@gmail.com*

Environmental and nutritional factors:

Among the environmental factors, many studies have focused on the relationship between how an infant is fed in the first year of life, especially breastfeeding, and how gluten is introduced (time and rate). In 2006, a review and met-analysis examined six case-control studies (3) that demonstrated the relationship between the duration of breast feeding and the reduced risk of CD. In these studies, there were limitations such as recall bias or the possibility of misclassification of controls or other risk factors, which may not consistent among infants. Another study suggested that greater attention should be paid to the introduction of gluten-free foods at 4 and 6 months of age, and it should be avoided in earlier stages (4).

In a double-blind trial, it was postulated that in individuals with HLA-DQ2 or DQ8 Positive who had the minimum first-degree dependency on CD, breastfeeding could reduce the frequency of CDs at the age of 3. However, the results showed that there was not any difference between the case study and placebo groups, suggesting that breastfeeding did not affect CD growth. In infants with HLA-DQ2 homozygote, CD was significantly higher than infants with other HLA (5). In two studies (5, 6), it was shown that neither the time of gluten introduction nor the time of breast feeding had a significant effect on the risk of CD. A maternal and child cohort study (7) is a population-based pregnancy cohort study aimed at linking CD development to prenatal parental factors and early nutritional measures as the age of breastfeeding. In this study, delayed gluten introduction was associated with the increased risk of CD and a positive association was found between breastfeeding for less than 12 months and CD risk compared to breastfeeding for a period of less than 6 months.

In general, both early feeding and the amount of gluten introduction during feeding and the risk factors of CD development are of paramount importance.

Epigenetics:

In point of epigenetics view, it can be classified into two main categories including twins studies and miRNAs function. In case of presence of phenotypic dissimilarity between two monoygotic (MZ) twins, it seems that epigenetics mechanisms are existing. In celaic disease, DNA methylation modifications, DNA methyltranferases inhibitors and histon changes are relevant to epigenetics issue.

To clarify the pertinent causes and analyze the involvement of environmental traits, the study of MZ twins are notified. In case of nuclear factors contribute to the organization of histone octamers. Polycomb group proteins, for instance EZH2, are being described to be connected to methylated genes. Methyl-CpG-binding domain (MBD) proteins are linked to methylated DNA and employ histone deacetylases (HDAC) and histone methyltransferases (HMT) to suppress transcription.

In order to inform the active histon modification, here is trimethylation of K4 of histone H3 or multiple acetylation of histone H3 and H4. Histone modification points allied to silencing are trimethylation of K9 and K27 of histone H3. Therefore, imprinted genes have stretched impact on phenotypic outcomes specially with focus on early embryonic development (8). Thereafter, researches showed that concordance rate in MZ twins are near to the ground in CD, suggesting the low impact of epigenetics modifications based on methylation (9).

In following, the role of microRNAs (miRNAs) cannot be neglected, since they occupy the gene expression as new modulators. MiRNAs impede with protein synthesis and control the posttranscriptional circumstances. Recently, miRNAs function in intestinal epithelium of

mice have informed. Among 453 families of miRNAs detected, mmu-miR-192 ascertained the most significant function in intestine. Subsequently, the role of Dicer protein proved over the differentiation and function of the intestinal epithelium (10).

The later researchers indicated the different miRNAs expression in intestinal biopsies of celiac children in comparison with control with nomination of miR-449 as a highly expressed marker on the contrary of NOTCH1 signaling (vital signaling pathway in preserving intestine homeostasis via goblet cells) (11). So as to inference of miRNA function, other researches verified the potential role in controlling treatment and management of CD and needs to be clarified in further researches (12, 13).

Diagnostics, from conventional pathology / serology to genetic testing

The first criteria for the diagnosis of Celiac Disease (CD) was established in 1970 by The European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN). The criteria were based on the certain histological changes in three small bowel biopsies, retrieval on a gluten-free diet, and relapse after reestablishment of gluten into the diet (14). There was regularly challenges in the clinical diagnosis of CD. From a side, the histological examinations failed to diagnose all of cases, in the other side the vast majority of CD patients were diagnosed with a substantial delay, because the diagnosis of celiac disease depends on the finding of distinctive small intestinal pathological abnormalities together with clinical or histologic enhancement on a gluten-free diet (15).

The first way to detect CD by means of an antibody test method was the detection of Anti-Gliadin anti-body (AGA) (16). Gliadins and glutenins are the two main components of the gluten fraction of the wheat seed (17). Anyhow, the AGA tests are no longer recommended for the diagnosis of classical CD (18).

Since 1990's reconsideration of ESPGHAN criteria which coined that in addition to just one biopsy the serological markers such as anti-endomysial (EMA) and anti-transglutaminase antibodies (t-TG) in addition to AGA, should be tested (19). The serologic testing of CD patients was boosted with the help of which the atypical forms of the disease can be diagnosed. This type testing improved the sensitivity and specificity of diagnosis and made the CD cases much easier to diagnose with accuracy. Accordingly, in this method, if a patient's blood assay reveals elevated antibody levels, the next step is an endoscopic biopsy (20).

In patients with villous atrophy, EMA antibodies of the immunoglobulin A (IgA) type can detect coeliac disease with a sensitivity and specificity of 90% and 99%, respectively. Serology for t-TG was primarily reported to have a higher sensitivity (99%) and specificity (>90%) (21).

A pitfall with serological detection of celiac disease is that there is an IgA deficiency in approximately 10% of the affected patients. This revenues that no IgA antibodies can be detected. However, this problem can be by-passed by not only determining the IgA antibodies but also accomplishment the slightly less specific IgG detection (22). The Immunopathology testing are not consistently the ultimate diagnostic method in several cases and there is some imprecise diagnosis here. So the implementing the molecular genetic testing predominantly HLA typing, which is a molecular test identifies certain antigens called Human Leukocyte Antigens in the blood and aids the immune system discriminate the body's individual proteins from foreign proteins, in combination with serologic testing (23). The t-TG and EMA testing are the most sensitive serum antibody tests, but as a negative HLA-DQ type excludes the diagnosis of coeliac disease, testing also for HLA-DQ2 or DQ8

maximizes sensitivity and negative predictive values. In facts, HLA typing is mostly an essential tool to classify individuals genetically susceptible to CD and identify high-risk individuals (24).

Even though the use of genetic risk prediction in clinics and mass population screening is still being argued in the scientific literature due to the ethical and social concerns, our risk prediction model for CD could by this time be utilized to help both in diagnostics and screenings strategies (25, 26).

Like other complex diseases, the early diagnosis in CD in newborns in terms of early intervention with treatment and prevention of durable complications such as developmental / growth disorders in child, seems pivotal (27). In diagnostic process of CD, HLA genotyping is already implemented widespread since it can help to exclude the disease in individuals with atypical CD but no HLA-DQ2/DQ8. By combining the HLA and non-HLA risks which will be clarified in next part, individuals could be better categorized into low-risk (< 0.1%), intermediate-risk (0.1% - 7%), and high-risk (> 7%) groups. Although the last two groups might have both negative and positive serology results. Once the serology testing results become positive, the follow up process can be closed according to the presence or lack of villous atrophy (26).

Lastly, the risk profiling could play an essential role in detection and follow up of individuals with non-specific and suggestive symptoms. In addition, Estimation of risk based on genetic profiling could increase precision by differentiating between two siblings (28). Based on proofs, genetic factors are not totally control the susceptibility, implying the environmental factors do play a correlated responsibility in the development of the condition.

Genetic Predisposition to Celiac Disease:

Recently, remarkable improvement has been succeeded in discovery of the genetic etiology of CD. Twin- and family-based studies evidently demonstrate a robust genetic predisposition to CD pathogenesis (29). The recurrence risk for siblings of CD patients to develop the disease is about 10%. Thus, the prevalence of CD in the general population is approximately 0.5%. The inheritance patterns also do not track Mendelian inheritance rule, which proposes that multiple genes are involved in pathogenesis and progression of CD.

The vast majority of genes involved in the pathogenesis of CD, provide instructions for making proteins in the immune system, cell signaling, oxidative stress, and apoptosis, which all play substantial role in tissue impairment in the gastrointestinal tract. It can explain the common pathways in autoimmunity and inflammation disorders. These genes can be sub-categorized into two main groups: HLA genes and non-HLA genes (30).

In HLA genes group, which its inheritance occurs as a certain haplotype, two of the most important CD genes are found on chromosomal region 6p21.3, and are so-called HLA-DQA1 and HLA-DQB1. Of course, it is important to mention that, Having these genes does not considered as the only cause of CD, because there are other genetic and/or environmental factors that also play a role in disease development. Nevertheless, the putative HLA gene variants are also found in 30 percent of the general population, and merely 3 percent of persons with the gene variants develop celiac disease (29). These two genes contain HLA class II alleles, which are to some extent unlike versions of the genes, exclusively in the HLA-DQ region on chromosome 6. HLA molecules are assumed to present gluten antigens to T-cells which is in charge for tissue impairment in CD patients (31). Allelic variants of the HLA-DQ locus, coding for the HLA-DQ2 and HLA-DQ8 molecules, contribute to about 40% of CD etiology (31).

Approximately more than 90% of CD patients carry DQ2.5 heterodimers, encoded by *DQA1*05* and *DQB1*02* alleles which are located on the same DR3-DQ2 haplotype. Also DQ8 molecules, encoded by *DQB1*03:02* commonly in combination with *DQA1*03* variant. Less frequently, CD occurs in individuals positive for the DQ2.x heterodimers and very infrequently in patients negative for these DQ predisposing markers. These combination of different allelic variants can generate the various HLA-DQ heterodimer proteins and affect the genetic predisposition to CD (32).

Occasionally the homozygosity / heterozygosity mode in the inheritance of these allele, can distinguish the clinical severity of disease. In other words, some alleles may have the dosage effect. For example in a study by Karinen et al, 2006 and also Al Toma et al, 2006, they have been suggested that being homozygote for *HLA-DQB1*02* allele might increase the severity of CD and increase the probability of developing refractory celiac disease, or Enteropathy-associated T-cell lymphoma (EATL) (33-35). There is also more or less evidences that suggest some alleles have modifier effects and protect the carrying-individuals against celiac. In a study carried out by Hadley D et al, 2015, the protective effects of HLA-DPB1*04:01 allele were proved. The presence of this allele may reduce the risk of t-TGs, an early marker of CD, among DR3-DQ2 children, approving that additional variants in the HLA region influence the risk for CD autoimmunity (36). Besides the aforementioned genes and alleles, there are also non-HLA genes related to the CD. More than 40 loci outside of the HLA region have been linked with CD. Furthermore, there are major geographical diversity in the prevalence and incidence of celiac disease that cannot be elucidated by only HLA genes (37).

With regard to their function, the non-HLA genes can be divided into two classes: the first group are the genes which have role in inflammation processes and the second group are contributed to the potential mucosal barrier. These genes include MYO9B, CTLA4, IL2, IL21, PARD3 and MAGI2. The non-HLA genes have only a modest effect and elucidate just a small proportion of familial disease risk (38). In 2005 Hunt KA et al demonstrated that a shared CTLA4 haplotype associated with CD. CTLA4 protein plays a crucial role in regulating T lymphocyte mediated inflammatory responses (39). In 2007 Wolters VM et al showed that The MYO9B gene is a strong risk factor for developing refractory celiac disease. MYO9B gene has instruction for coding a GTPase activating protein, which is involved in epithelial cell cytoskeletal organization (40-42). Also the Wapenaar MC et al ,research team in 2008 illustrated the association between major variants (SNP) in PARD3 and MAGI2 genes and CD in Dutch population. These tow genes encodes proteins which have role in cellular tight junction and might affect epithelial barrier function, thus the pathogenic variants within them considered as risk factor for CD and ulcerative colitis (43).

In order to identify the new susceptible genes and variants in CD there are different genetic approach which are used by human genetics researchers. These strategies include from the narrow–spectrum tool like single candidate gene analysis to the widest techniques as genome wide association studies (GWAS). In between there are also some approaches such as linkage study which explores the biased transmission of certain chromosomal loci through the whole genome and it is tracked by fine-mapping of linkage regions encompassing several positional candidate genes (30). GWAS is also a comprehensive tool to analyze the whole genome in terms of existence of polymorphic variants known as Single Nucleotide Polymorphisms (SNP) within or outside of coding region. The output of GWAS studies as

risk variants occasionally improve the risk assessment in CD patients (44).

Using GWAS strategies which are implemented in big international cohort studies, numerous polymorphic loci are identified in addition to the main susceptibility genes. These polymorphic loci are in physical association of many genes with significant statistical scores (37). Last but not least, such genetic approaches can provide invaluable information concerning risk variants and can be utilized as disease risk predictors, even if the complete catalogue of susceptibility variants are still unidentified (45).

Gene category	Gene name	Chromosome location	Function	Identification approach
HLA genes	HLA-DQ (include several polymorphic allelic variants)	6p21.3	Anti-gene presentation in immune system	Miscellaneous
Non-HLA genes	CTLA4	2q33	Regulation of T-cell response, activation and proliferation	Candidate gene
	ICOS	2q33.2	Ig class-switching	
	IL2/IL21	4q26-q27	Regulation of the proliferation of T, B and NK lymphocytes	GWAS
	MYO9B	19p13.1	Cytoskeleton remodeling	Linkage Analysis and GWAS
	PARD3	10p11.21	Cellular tight junction	GWAS
	MAGI2	7q21.11		

Present & Prospective challenges in CD researches:

Over the two recent decades, Human Genetics Researches in the matter of widespread identification of either causal or susceptibility variants across the genome rather than single genes, have had significant successes. This achievement is revealed by the large number of genome analysis approaches like GWAS that have broadened our understanding of the biological pathways involved in disease pathogenesis and development.

Particularly in GWA studies the unavoidable demand for larger international cohorts with significant ethnic diversity is noticeable. Also, implementing the next-generation sequencing (NGS) in terms of identification of rare variants instead of common variants may serve as a paradigm towards the better understanding of disease molecular pathology hence bridging from bench to bed more efficiently (46, 47). Moreover, one of the most important challenge of future regarding CD is research into risk prediction models and functional analyses (48). For instance the improvement of testing of non-HLA variants such as SNPs additional to HLA genes can increase the risk prediction in CD which are not properly diagnosed and therefore remain untreated patients (49). At present, the only treatment for CD is gluten free diet, and there is an actual demand for alternative therapies in future. Furthermore, Establish of *in vitro* and *in vivo* models of CD in order to deeper understanding the pathogenesis of CD is essential which in this matter, there extensive achievements are now exist. The *in vitro* models

including an epithelial layer consistent with celiac disease include Caco-2 and IEC-6 epithelial cell lines, Monocytes / Dendritic cells and APC/T Cell Mixes as model for investigating the gluten responsiveness. Also *in vivo* ones are spontaneous animal models, induced models and transgenic mouse models (50).

Conflict of Interest:

Authors declare no conflict of interest with any person or organization.

Acknowledgement:

Imam Reza International University Mashhad, Iran is acknowledged for support to complete this literature reivew.

References:

1. Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, et al. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. Annals of medicine. 2010;42(8):587-95.
2. Rostom A, Dubé C, Cranney A, Saloojee N, Sy R, Garritty C, et al. The diagnostic accuracy of serologic tests for celiac disease: a systematic review. Gastroenterology. 2005;128(4):S38-S46.
3. Akobeng AK, Ramanan AV, Buchan I, Heller RF. Effect of breast feeding on risk of coeliac disease: a systematic review and meta-analysis of observational studies. Archives of disease in childhood. 2006;91(1):39-43.
4. Norris JM, Barriga K, Hoffenberg EJ, Taki I, Miao D, Haas JE, et al. Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease. Jama. 2005;293(19):2343-51.
5. Vriezinga SL, Auricchio R, Bravi E, Castillejo G, Chmielewska A, Crespo Escobar P, et al. Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med. 2014;2014(371):1304-15.
6. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Tonutti E, Amarri S, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. New England Journal of Medicine. 2014;371(14):1295-303.
7. Størdal K, White RA, Eggesbø M. Early feeding and risk of celiac disease in a prospective birth cohort. Pediatrics. 2013;132(5):e1202-e9.
8. Ballestar E. Epigenetics lessons from twins: prospects for autoimmune disease. Clinical reviews in allergy & immunology. 2010;39(1):30-41.
9. Selmi C, Mayo MJ, Bach N, Ishibashi H, Invernizzi P, Gish RG, et al. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. Gastroenterology. 2004;127(2):485-92.
10. McKenna LB, Schug J, Vourekas A, McKenna JB, Bramswig NC, Friedman JR, et al. MicroRNAs control intestinal epithelial differentiation, architecture, and barrier function. Gastroenterology. 2010;139(5):1654-64. e1.
11. Capuano M, Iaffaldano L, Tinto N, Montanaro D, Capobianco V, Izzo V, et al. MicroRNA-449a overexpression, reduced NOTCH1 signals and scarce goblet cells characterize the small intestine of celiac patients. PloS one. 2011;6(12):e29094.
12. Vaira V, Roncoroni L, Barisani D, Gaudioso G, Bosari S, Bulfamante G, et al. microRNA profiles in coeliac patients distinguish different clinical phenotypes and are modulated by gliadin peptides in primary duodenal fibroblasts. Clinical Science. 2014;126(6):417-23.
13. Richmond C, Breault D. Regulation of gene expression in the intestinal epithelium. Prog Mol Biol Transl Sci 2010; 96: 207-29; PMID: 21075346.
14. Meeuwisse G. Diagnostic critera in coeliac disease. SCANDINAVIAN UNIVERSITY PRESS PO BOX 2959 TOYEN, JOURNAL DIVISION CUSTOMER SERVICE, N-0608 OSLO, NORWAY; 1970.
15. Matthias T, Pfeiffer S, Selmi C, Gershwin ME. Diagnostic challenges in celiac disease and the role of the tissue transglutaminase–neo-epitope. Clinical reviews in allergy & immunology. 2010;38(2-3):298-301.
16. Volta U, Cassani F, De Franchis R, Lenzi M, Primignani M, Agape D, et al. Antibodies to gliadin

in adult coeliac disease and dermatitis herpetiformis. Digestion. 1984;30(4):263-70.
17. Bethune MT, Khosla C. Parallels between pathogens and gluten peptides in celiac sprue. PLoS Pathog. 2008;4(2):e34.
18. Armstrong D, Don-Wauchope AC, Verdu EF. Testing for gluten-related disorders in clinical practice: the role of serology in managing the spectrum of gluten sensitivity. Canadian Journal of Gastroenterology and Hepatology. 2011;25(4):193-7.
19. Walker-Smith J, Guandalini S, Schmitz J, Shmerling D, Visakorpi J. Revised criteria for diagnosis of coeliac disease. Archives of Disease in Childhood (United Kingdom). 1990.
20. Rashtak S, Ettore M, Homburger H, Murray J. Combination testing for antibodies in the diagnosis of coeliac disease: comparison of multiplex immunoassay and ELISA methods. Alimentary pharmacology & therapeutics. 2008;28(6):805-13.
21. van der Windt DA, Jellema P, Mulder CJ, Kneepkens CF, van der Horst HE. Diagnostic testing for celiac disease among patients with abdominal symptoms: a systematic review. Jama. 2010;303(17):1738-46.
22. Gregor J, Alidina DS. Celiac disease. Evidence-based Gastroenterology and Hepatology, Second Edition. 2004:169-78.
23. Kaukinen K, Partanen J, Mäki M, Collin P. HLA-DQ typing in the diagnosis of celiac disease. The American journal of gastroenterology. 2002;97(3):695-9.
24. Hadithi B, Crusius J, Bloemena E, Kostense P, Meijer J, Mulder C, et al. Accuracy of Serologic Tests and HLA-DQ Typing for Diagnosing Celiac Disease.
25. Mearin ML, Ivarsson A, Dickey W. Coeliac disease: is it time for mass screening? Best practice & research Clinical gastroenterology. 2005;19(3):441-52.
26. Romanos J, Wijmenga C. Predicting susceptibility to celiac disease by genetic risk profiling. Annals of Gastroenterology & Hepatlogy. 2010;1:1-9.
27. Brigic E, Hadzic D, Mladina N. Early and Correct Diagnosis of Celiac Disease in the Prevention of Growth Disorders and Child Development. Materia socio-medica. 2012;24(4):242.
28. Dubé C, Rostom A, Sy R, Cranney A, Saloojee N, Garritty C, et al. The prevalence of celiac disease in average-risk and at-risk Western European populations: a systematic review. Gastroenterology. 2005;128(4):S57-S67.
29. Louka A, Sollid L. HLA in coeliac disease: unravelling the complex genetics of a complex disorder. Tissue antigens. 2003;61(2):105-17.
30. Zhernakova A, Wijmenga C. HLA and Non-HLA Genes in Celiac Disease. Frontiers in Celiac Disease: Karger Publishers; 2008. p. 32-45.
31. Sollid LM. Coeliac disease: dissecting a complex inflammatory disorder. Nature Reviews Immunology. 2002;2(9):647-55.
32. Megiorni F, Pizzuti A. HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: practical implications of the HLA molecular typing. Journal of biomedical science. 2012;19(1):88.
33. Ploski R, Ek J, Thorsby E, Sollid LM. On the HLA-DQ (α1* 0501, β1* 0201)-associated susceptibility in celiac disease: A possible gene dosage effect of DQB1* 0201. HLA. 1993;41(4):173-7.
34. Karinen H, Kärkkäinen P, Pihlajamäki J, Janatuinen E, Heikkinen M, Julkunen R, et al. HLA genotyping is useful in the evaluation of the risk for coeliac disease in the 1st-degree relatives of patients with coeliac disease. Scandinavian journal of gastroenterology. 2006;41(11):1299-304.
35. Al–Toma A, Goerres MS, Meijer JW, Peña AS, Crusius JBA, Mulder CJ. Human leukocyte antigen–DQ2 homozygosity and the development of refractory celiac disease and enteropathy-associated T-cell lymphoma. Clinical Gastroenterology and Hepatology. 2006;4(3):315-9.
36. Hadley D, Hagopian W, Liu E, She J-X, Simell O, Akolkar B, et al. HLA-DPB1* 04: 01 protects genetically susceptible children from celiac disease autoimmunity in the TEDDY study. The American journal of gastroenterology. 2015;110(6):915-20.
37. Sharma A, Liu X, Hadley D, Hagopian W, Liu E, Chen W-M, et al. Identification of non-HLA genes associated with celiac disease and country-specific differences in a large, international pediatric cohort. PloS one. 2016;11(3):e0152476.

38. Romanos J, Rybak A, Wijmenga C, Wapenaar MC. Molecular diagnosis of celiac disease: are we there yet? Expert opinion on medical diagnostics. 2008;2(4):399-416.
39. Hunt KA, McGovern DP, Kumar PJ, Ghosh S, Travis SP, Walters JR, et al. A common CTLA4 haplotype associated with coeliac disease. European Journal of Human Genetics. 2005;13(4):440-4.
40. Wolters VM, Verbeek WH, Zhernakova A, Onland–Moret C, Schreurs MW, Monsuur AJ, et al. The MYO9B gene is a strong risk factor for developing refractory celiac disease. Clinical Gastroenterology and Hepatology. 2007;5(12):1399-405. e2.
41. Post PL, Tyska MJ, O'Connell CB, Johung K, Hayward A, Mooseker MS. Myosin-IXb is a single-headed and processive motor. Journal of Biological Chemistry. 2002;277(14):11679-83.
42. Matter K, Balda MS. Signalling to and from tight junctions. Nature reviews Molecular cell biology. 2003;4(3):225-37.
43. Wapenaar MC, Monsuur A, van Bodegraven A, Weersma RK, Bevova M, Linskens R, et al. Associations with tight junction genes PARD3 and MAGI2 in Dutch patients point to a common barrier defect for celiac disease and ulcerative colitis. Gut. 2007.
44. Garner C, Ahn R, Ding YC, Steele L, Stoven S, Green PH, et al. Genome-wide association study of celiac disease in North America confirms FRMD4B as new celiac locus. PloS one. 2014;9(7):e101428.
45. Wray NR, Goddard ME, Visscher PM. Prediction of individual genetic risk of complex disease. Current opinion in genetics & development. 2008;18(3):257-63.
46. Balasopoulou A, Stanković B, Panagiotara A, Nikčevic G, Peters BA, John A, et al. Novel genetic risk variants for pediatric celiac disease. Human Genomics. 2016;10(1):34.
47. Siu H, Zhu Y, Jin L, Xiong M. Implication of next-generation sequencing on association studies. BMC genomics. 2011;12(1):322.
48. Wijmenga C, Gutierrez-Achury J. Celiac disease genetics: past, present and future challenges. Journal of pediatric gastroenterology and nutrition. 2014;59:S4-S7.
49. Romanos J, Rosén A, Kumar V, Trynka G, Franke L, Szperl A, et al. Improving coeliac disease risk prediction by testing non-HLA variants additional to HLA variants. Gut. 2014;63(3):415-22.
50. Stoven S, Murray JA, Marietta EV. Latest *in vitro* and *in vivo* models of celiac disease. Expert opinion on drug discovery. 2013;8(4):445-57.

