GENETICALLY MODIFIED RODENT MODELS AND CELIAC, NON-CELIAC GLUTEN SENSITIVITY: A MINIREVIEW

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SUMMARY

Celiac disease (CD) is a disorder that affects both children and adults. Over the few last decades, several new atypical cases have been identified through improved diagnostic tools. On the other hand, the onset of CD at a later age, including atypical CD forms whose clinical picture overlaps with other autoimmune diseases, shows that currently there are several unknown gene mutations, which could be responsible for the disease development. Non-celiac gluten sensitivity (NCGS) is entity included by the ingestion of gluten leading to intestinal, or extraintestinal symptoms that improve once the gluten is removed from the nutrition. In this article relationships between genetically modified rodent animals with previously unknown multiple organ changes and CD, respectively NCGS are reviewed. Relationships between the small bowel histological changes and other organs pathology are discussed. Results of research document that changes have similar genetic background and can develop to serious autoimmune systematic diseases, including small bowel inflammation resembling atypical CD or NCGS. These may have extra-intestinal symptomatology but without a clear explanation of causes and differences in their manifestations. Research on animal models helps to discover links between several disorders associated with gastrointestinal damage. New methods based on individual gene mutations can help in atypical adult CD and NCGS recognitions in the future.

Key words: animal models, celiac disease, gene mutation, mouse, non-celiac gluten sensitivity, prevention, rat, small bowel, gluten, pathology

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INTRODUCTION

Malabsorption can result from a large variety of organic and functional disorders and refers to impaired nutrient absorption. They are classified from pathological point of view to primary and secondary. Small bowel is the most important organ of absorption, so small bowel biopsy is therefore important in the evaluation of gluten related diseases. Celiac disease (CD) is a well-known disorder, which affects both children and adults. Relationships between gluten and disease have been proven in the past. Historically, the CD was a particularly rare disorder, but today approximately 1/100 to 1/200 people are affected worldwide (1). It has been more than 25 years since the article about the celiac iceberg was first published (2). The assumption about higher diagnostics was fulfilled. Nevertheless, diagnostics still poses one of the main problems, and not only in developing countries. So it is no surprise that CD is either not diagnosed or is diagnosed only later in life (3). At first, it was thought that insufficient attention was given to CD, or it was simply forgotten during routine clinical practice. It was already known at this time that CD could also manifest itself extra-intestinally, or in atypical forms. Together with the improvement and introduction of more sensitive tests, several new associations between CD and other diseases were found. On the other hand, it was not entirely clear

if they were results of gluten intolerance, or they were results of prolonged untreated CD. It can be stated that the typical CD form is satisfactorily recognised today, but atypical CD forms have been signed under insufficient diagnostics (4). Non-celiac gluten sensitivity (NCGS) is characterized by intestinal and extra-intestinal symptoms related to the ingestion of gluten-containing foods in the absence of celiac disease and wheat allergy. The mechanisms that determine symptoms in NCGS after gluten ingestion are still unknown, but it is evident that there are marked differences with CD (5). Currently there are no typical biomarkers, it seems like NCGS will be more common than CD (6). NCGS symptoms are highly variable and final diagnosis can be established only by excluding CD. In this article, we do not want to point out the differences in diagnosis between CD, NCGS or wheat allergy, including differences in diagnosis between individual continents or even between individual states. The objective of this article is to show and discuss the relationships between genetics and individual diseases, including small bowel changes resembling CD, or NCSG changes on genetically modified rodent models with previously unknown multiple organ changes. Today, there are still many unrecognised autoimmune disorders, including atypical CD and also NCGS cases. The target of the article will be relationships responsible for the onset of several disorders, including late-onset CD, NCGS, and also what was done to improve CD diagnostics and vice versa. Identification of specific mutations in individual genes can be responsible for triggering the manifestations of CD, NCGS at a later age. In this sense, biomedical research will play a crucial role in the diagnostic algorithms of the future, including the importance of predictive and preventive medicine of several diseases.

CD Diagnosis Improvement

Several factors were involved in the improvement of CD diagnostics. Many other articles have discussed this, so only major contributions will be briefly outlined. First, a gastroenterologist's primary role in CD diagnosis required changes in modifications to the diagnostic algorithm since they are responsible for CD diagnosing (7). Second, adjustment of the diagnostic criteria by reducing repeated biopsies, and the introduction of serological tests significantly contributed to improvements in CD diagnosis. In the first criteria for CD, three duodenal biopsies were required for the diagnosis. The first was taken initially with gluten, the second after a gluten-free diet, and the last after a gluten challenge (8). No changes were made to this for a long time. After, taking only two biopsies was suggested with a discussion about what time interval should be between these biopsies. The revised criteria for CD diagnosis suggested a serological test and one biopsy, but there is still discussion whether a biopsy is important, and from which part of the small bowel it should be taken (9). In accordance with new criteria, diagnosis can be stated in children without biopsy if there are highly positive tissue transglutaminase antibodies (> 10 times the upper limit of normal TGA-IgA) with endomysial antibodies (EMA-IgA) positivity in a second blood sample (10). From this, it can be stated that reducing the number of biopsies and replacing them with several well-known non-invasive serological tests played an important role in CD diagnosis (11). Small bowel biopsy evaluation system from patients with CD describes five histologic lesions, which are well readable from H&E-stained and CD3-stained sections. However, there were some problems with other tests interpretation. Individual laboratories had different interpretations of results. For example, what was considered an intense immunofluorescence signal in one laboratory, was moderately strong in other laboratories, or marked on a scale in a third laboratory. The procedure needed to be unified and therefore, it was necessary to train staff and accredit diagnostic laboratories. In this process, commercial kits began to be used, which eliminated mistakes from the handmade tests used at the time. Finally, gluten-free diet popularisation shed more light about CD to professionals and the public, because until less-known disease came to the attention of the public (12).

CD and NCGS Diagnosis

As the results of several clinical studies showed, atypical CD is clinically manifested in broad symptomatology. How to search for these forms is currently intensively discussed in the scientific literature. However, less attention is given to the causes that signify the onset of CD at a later age. In this sense, it is generally accepted that the genetic basis plays an important role in CD pathology. Today, it is well known that the human leukocyte antigen (HLA) genes determine the main genetic CD predisposition, and their antigens are present on the surface of the immune system cells. HLA-DQ2 and HLA-DQ8 are present in 99% of CD cases (13). On the other hand, several people without CD were identified with these genes in the population, so these genetic phenotypes do not explain the pathogenesis entirely (14). Generally, up to 95% of patients with CD are positive for HLA-DQ2, and the remaining 5% are positive for HLA-DQ8 haplotypes (15). There are some differences in HLA positivity in European, Chinese, African, Tunisian, Iranian populations, including differences in CD patients (16). The results of a study by Kuja-Halkola et al. (17), based on a population-representative sample of 107,912 twins, documents that CD is characterized by high heritability, but it also suggests that non-shared environmental factors may be of importance to CD development. That means that HLA has only a moderate impact on estimates of heritability. Regarding non-HLA genes, recently several gene loci associated with increased CD susceptibility have been successfully identified. For example, Van Belzen et al. (18) identified a significant linkage between CD and chromosome regions 19p13.1 and 6q21-22. Another study analysed 778 CD individuals and 1,422 controls, documenting that outside the HLA region, the most significant findings (rs13119723; $P = 2.0 \times 10^{(-7)}$) were in the KIAA1109-TENR-IL2-IL21 linkage disequilibrium block (19). In another study, Sharma et al. (20) found influences between CD and 8 non-HLA regions not previously associated with CD. Romanos et al. (21) investigated whether CD risk prediction could be improved by adding non-HLA-susceptible variants to common HLA testing. The results documented that predicting risk with 57 additional non-HLA variants improved the identification of potential CD patients. It can therefore be stated that non-HLA risk alleles play a role in CD pathology, which can be screened for in an improved method of identifying of high-risk individuals (22). Regarding NCGS, there is still absence of clear-cut diagnostic criteria. Multimodal pragmatic approach combining findings from the clinical history, symptoms, serological tests, and histological finding are required in order to reach an accurate diagnosis (23). In accordance with

Salerno Experts criteria a full diagnostic procedure should assess the clinical response to the gluten-free diet and measure the effect of a gluten challenge after a period of treatment with the gluten-free diet, including clinical evaluation using a modified version of the gastrointestinal symptom rating scale (24). If the significance of the above facts is considered, it is obvious that CD including NCGS can be underdiagnosed or misdiagnosed conditions in routine clinical medicine practice. Today, it is still not known whether other gene mutations influence CD, NCGS development, or if there are influences from other systematic disorders. Searching for individual gene mutations in autoimmune disorders are challenge for modern biomedical research. Although the human genome has already been sequenced, the phenotypical significance of several genes is still unknown. The International Mouse Phenotype Consortium (IMPC) has developed a program to generate and phenotype mouse mutants for every gene in the mouse genome (25). Their main objective is to create more than 20,000 strains – each with one specific deactivated gene and continue to expand the view of the genomic landscape for multiple disease loci. Based on the assumption that the mouse genome is >95% identical to the human genome, results can be applied to drug discovery and gene therapy in the future. Much effort goes into these therapies, to give hope to the patients in need. This also applies to CD, NCGS but it is necessary to know the full genetic background of the diseases first.

Animal Models and Diseases

Several animal models were developed to study diseases changing pathophysiology. There are some experiences with dogs, monkeys, non-transgenic rodents, and finally genetically engineered rodents in connections with CD (26). All models were based on well-known genetic conditions, or on affecting the integrity of the small bowel mucosa. On the other hand, it is well known that autoimmune disorders have strong genetic background. So it can be hypothesized that in their pathophysiology plays some role also other, currently unknown gene mutations. Genetically modified rodent models are based on systematically identifying the function of every protein-coding gene in the animal genome. The basic research centres around the world are equipped with comprehensive collections of tools for the physiological and morphological assessment of experimental mice and rats in a controlled specific pathogen-free environment. Many novel genetically modified mice and rats are generated using state-of-the-art technologies. Mice or rats undergo several screenings, and they are subsequently delivered to the histopathology department for final investigation, consisting of two steps. The first part is a macroscopic investigation, including measurements, organ sampling, which takes place during the necropsy. Following this, the second part consists of a histological investigation of the sampled organs. Each cohort contains prescribed mice and organs from selected female and male animals, including cytological smears (27). The produced report contains results and comparison of individual measurements, summary of macroscopic pathology, following histological description of the investigated samples and final diagnosis, which was done by an animal pathologist (28). The work is coordinated with the IMPC and its objective to generate a null mutant and undertake broad-based phenotyping for every gene in the mouse genome. Results are then available to be used in future research dealing with disease diagnostics and treatment. Several genes have been included in this screening, without prior knowledge of their importance to specific diseases or phenotypes. Many individual gene mutations have been found to influence several disorders using these methods (29). Hypospermatogenesis is the most common histological change in investigated mutant mice. In several cases, relationships between the male reproduction system and histological changes in the thyroid gland were identified. Histological changes in the reproductive organs (spermatocytic arrest, testicle atrophy, aspermia, and reduced secretion in other reproductive glands – prostate, seminal vesicles) were found in combination with thyroid gland follicular hyperplasia, follicular degeneration, follicular cell necrosis, or also follicular cell dysplasia. Part of the results document small bowel changes. Here especially increased lymphocytes in mucosa with preserved villous architecture, or with elongated crypts are present. In other cases, mild or developed enteritis, including other mucosal changes like oedema, or increasing numbers of eosinophils are visible. In some individual cases miniature small and large bowels ulceration are in combination with the above-mentioned pathologies visible. These results document that several diseases can have the same genetic background, and after the stress of the system the pathology begin to show. This may also apply to inflammatory bowel diseases, including late-onset CD or NCGS. Today, it is clear that there are more genes influencing CD and NCGS predisposition to varying degrees of strength and significance. However, it is not known how these genes relate to other diseases and if mutations in multiple genes are required to trigger the disease or even groups of disorders. This may be important in the pathophysiology of several diseases. It is estimated that many CD cases still go undiagnosed, especially among the elderly and in patients with atypical clinical presentations, yet the frequency of missed CD diagnoses is on the rise (30). The National Institute of Health Consensus Development conference documented an increase in CD diagnosed patients of up to 12% after targeted screening (31). Many cases were asymptomatic, and for example, 7% of cases presented male infertility. However, this does not apply only to males. Singh et al. (32) document that CD is more prevalent in women with infertility, so when the cause for infertility is unknown, it is advisable to conduct CD tests (33). Furthermore, another study described associations between CD and endocrine diseases, including diabetes and autoimmune thyroid syndromes (34). Elfstrom (35) monitored 14,000 individuals with CD and documented statistically significant associations with an increased risk of liver disease, Addison's disease, thyroid disease, autoimmune heart disease, leukaemia, and lymphoma. On the other hand, symptoms of NCGS are similar to other gluten-related diseases, irritable bowel syndrome and Crohn's disease (36). According to the Losurdo et al. (37), the most commonly autoimmune disorders associated with NCGS are Hashimoto thyroiditis, dermatitis herpetiformis, psoriasis, and also rheumatologic diseases. These data correspond with genetically modified rodent models experimental results, leading to our hypothesis that several disorders can display a common genetic predisposition. Although this article does not show causal relationships between individual genes, CD, NCGS and other described disorders, factors that may be responsible for insufficient autoimmune diseases diagnostics are discussed. It is indicated why the atypical CD forms are unrec-

ognized, and why this is problematic in clinical practice. This statement applies also to other inflammatory bowel diseases, which are diagnosed in adult or elderly patients. As mentioned above, in NCGS gluten intake is the primary cause of pathological changes in several organs. Today, there are gluten-related disorders with poorly understood pathogenesis, but only studies performed on large sample sizes, with the inclusion of control groups, will be able to clearly and seriously reply to questions about their pathology. This applies also to their genetic background research. Even though CD pathophysiology with a strong immune background and small bowel pathology is well understood, several atypical cases are still not diagnosed, or they are diagnosed too late in life. Among the above-mentioned things, it is also today supposed that late onset CD could be attributed to environmental factors that participate in the loss of tolerance to gluten (38). This was confirmed also on rodent models. Mice expressing human HLA develop innate immune activation following sensitization to gliadin (39). In a recently published article, Abadie et al. (40) generated a mouse model that mimicked CD. After 30 days of diet with gluten, 75% of these mice developed small intestinal tissue destruction, but following a gluten-free diet, small bowel remission was observed, returning to normal morphology. Such models will definitely shed more light on the relationships between gluten, genetics and the immune responses that drive CD development. Today it is already well known that CD may have a later onset and extra-intestinal symptomatology, but without a clear explanation of causes and differences in their manifestations. The onset of multiple disorders can be connected and initiated by individual gene mutations. So, it should not be neglected that other gene mutations may also play a role in the CD or NCGS pathogenesis. Individual gene mutation identification could provide at least partial answers to these questions. It is obvious that the future of targeted biomedical, genetic research will improve knowledge of CD, NCGS pathophysiology, including clarifying links between gene mutations, and other gluten related disorders. Research on genetically modified rodent models with one specific deactivated gene will help to discover links between several disorders associated with bowels damage, including atypical CD and NCGS.

CONCLUSION

Research on individual gene mutations will play an important role in the future of gluten and autoimmune related disorders diagnostic algorithms, including improving CD, NCGS diagnosis, CD, NCGS classification and typology, and finally in CD, NCGS treatment. Until mutations in the individual genes responsible for the disease are identified, cases of atypical CD, NCGS will still go unrecognised, misdiagnosed and also underdiagnosed.

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Conflict of Interests

None declared

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