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Review

Role of genetics in pediatric rheumatology

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Abstract

Pediatric rheumatology includes autoinflammatory monogenic diseases, autoinflammatory multifactorial diseases with complex inheritance, and diseases with uncertain clinical diagnosis or undefined conditions, even though they show signs of autoinflammation. Most of these diseases are systemic; it is important to diagnose patients promptly and definitively and to select proper treatment options based on the diagnoses. Clinical observation and acute-phase responses are usually sufficient for diagnosis; however, genetic analyses can provide supportive data for definite diagnosis and treatment, especially for rare monogenic diseases. As for multifactorial autoinflammatory diseases, susceptibility genes, and factors involved in the etiopathogenesis have not been fully identified. It is possible to identify disease genes and novel diseases, and lead to new treatment options by gene mapping studies and high-throughput screening strategies for multifactorial diseases and conditions with uncertain clinical characteristics. In this review, we discuss the three groups of autoinflammatory diseases and role of genetics in their diagnosis.

Keywords: Diagnosis, genetics, pediatric rheumatology

Introduction

In Pediatric Rheumatology outpatient clinics, early diagnosis of different types of diseases, which are mostly systemic, and accurate treatment in association with the diagnosis is very important. Although diagnoses are basically made through clinical observation and examinations of acute-phase responses, genetic diagnosis may be supportive in diagnosis and directive in treatment in some of these diseases. This is especially important in terms of rare monogenic diseases (hereditary autoinflammatory diseases), which constitute a part of inflammation-based diseases that occur in childhood. As explained in detail below, some rare monogenic diseases including Familial Meditarenean fever (FMF) are observed more frequently in our country because of both our geography and increased consanguineous marriages. In addition to these hereditary diseases, there are also multifactorial autoinflammatory diseases that are inherited with complex-type models and occur with interactions of genetic and environmental factors, including systemic juvenile idiopathic arthritis. Altough susceptibility genes and defects of these genes related with diseases have been identified, the factors involved in the etiopathogenesis are still not fully known. Finally, there are also patients who have autoinflammatory signs, but whose clinical diagnoses cannot be made clearly or who show familial inheritance but are not phenotypically compatible with diseases described to date. For this group of diseases, early diagnosis and potential effective treatment options are possible by specification of diseases that have not been previously described, and the related genetic mutations based on the type of familial inheritance and presence and number of relatives with and without disease. In this review, the place and importance of genetic studies in pediatric rheumatic diseases, which are observed relatively frequently in our country, are explained under three titles:

1) The genetic basis of monogenic autoinflammatory diseases (MAD) and the importance of genetic diagnosis

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2) Known susceptibility genes in frequently observed diseases with complex inheritance

3) Definition of new genes and diseases in familial cases with an unclear clinical picture

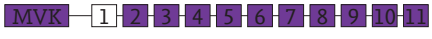
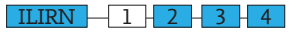
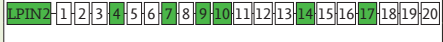
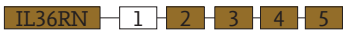
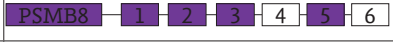
1. The genetic basis of monogenic autoinflammatory diseases and the importance of genetic diagnosis

The detection of single-gene mutations enabled description of a group of genetic autoinflammatory diseases characterized by recurrent fever and inflammation. These diseases are characterized through clinical findings including excessive inflammasome activation, recurrent fever episodes, eruption, urticarial rash, serositis, lymphadenopathy, and arthritis, which occur as a result of disorders of the innate immune system. These diseases are differentiated from autoimmune diseases with occurence of inflammatory episodes in the absence of autoantibody production and autoreactive T cells. Although these diseases generally occur in childhood, onset may rarely be observed in adulthood (1). Monogenic autoinflammatory diseases are classified in different groups according to clinical properties and pathogenesis (2). The diseases described in this scope include FMF, tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), mevalonate kinase deficiency/hyperimmunoglobulin D syndrome (MKD-HIDS), NLRP12- related syndrome (NLRP12AD), Blau syndrome, pyogenic arthritis, pyoderma gangrenosum and acne syndrome (PAPAs), early-onset sarcoidosis (EOS), Majeed syndrome (MS), interleukin-1 receptor antagonist deficiency (DIRA), cryopyrin associated periodic syndromes (CAPS), IL-36 receptor antagonist deficiency (DITRA), CARD14-mediated pustular psoriasis (CAMPS) and chronic atypical neutrophilic dermatosis, lypodistrophy, and elevated temperature (CANDLE). The genes, mutations, and inheritance models associated with these diseases are shown in Figure 1. In addition, the genetic characteristics and diagnoses of those that are observed relatively frequently in our population are explained in more detail below. The most reliable mutation detection method used in genetic diagnosis laboratories for most of these diseases includes reproduction of the encoding regions in the disease-related gene (exons shown in Table 1) in DNA samples obtained from patients using polymerase chain reaction (PCR) for Sanger sequencing, automated capillary DNA sequencing (including ABI), and comparison of the obtained DNA sequences (Electropherogram) with healthy controls. Although PCR and reverse-hybridization-based tests are also used routinely for some of these diseases in our country, these tests may give erroneous results (3). In addition, the strip test generally used for FMF can detect only 12 mutations and may occasionally miss the other mutations found in exons 2-3-5 and 10. Another method is next-generation sequencing (NGS). All candidate gene regions and sequences of the patient's one or more genes including the encoding and non-encoding parts are obtained with this method. Although this is the most important advantage of this method, evaluation of the results in the context of bioinformatics and subsequent genetic counseling require advanced expertise. As a result of this type of analysis, approximately 300-500 variants have been observed without bioinformatics-based filtering on an average gene (with a length of 15 kb). Subsequently, the number of variants may decrease from 30-50 to 5-10 depending on the measurements if the variants are in the encoding or non-encoding regions, if they change amino acids and the prevalences in the population. A comparison of the advantages and disadvantages of genetic diagnostic methods used for these types of disease is shown in Table 2 (4-6). Including all this information and providing a detailed explanation of the potential relationship regarding the mutation/variant with the phenotype in genetic diagnosis reports that are presented to physicians is important.

1.1. Familial Meditarenean fever

Familial Mediteranean fever, which is the most common MAD in our community, is associated with inflammation in the peritoneum, synovium, pleura, and rarely the pericardium, mostly in association with fever. The prevalence of FMF in the Turkish population is 1/1000, though this increases to 1/395 in internal regions. The *MEFV* gene, which is associated with FMF, is found on the 16p13.3 region and is composed of 10 exons (7). The *MEFV* gene, which is specifially expressed in neutrophils, eosinophils, monocytes and dendritic cells, encodes the 781–amino-acid pyrin/marenostrin protein. *MEFV* gene mutations lead to an increase in cytokine synthesis, NF-κB activation, and inhibition of apoptosis. These changes in the inflammatory mechanism leads to the pathogenesis of FMF (8). Although FMF shows an autosomal recessive inheritance, cases showing dominant or complex heterozygous inheritance or carrying no mutation have been reported. One hundered seventy-one variations that have been associated with FMF have been found on the *MEFV* gene. Current mutations and variations for all MADs are found in the *InFevers* database (9). M694V, M680I, V726A, M694I, and E148Q, which are among the mutations found in the 2nd, 3rd, and 10th exons, constitute 70% of the variations that have been associated with the disease. The high carrier rates for *MEFV* mutations

Table 1. Commonly used methods in the genetic diagnosis of hereditary autoinflammatory diseases

Disease	Gen	Exon/Mutation							
FMF		2	R143P, E148Q, R151S, E167D, T177I, S179I, P180R, G196W, S242R, E225D, T267I, A268V, P283L, A289V, E299G			3	T309M, R354W, P369S, R408Q		
		5	V496L, H478Y, F479L, I506V, G514E			10	P646L, L649P, D661N, G678E, M680I/L, T681I, Y688X, M692del, M694L/V/I/del, K695R/S, V704I, L709R, R717S, V726A, F743L, A744S, S749C, R761H, I772V		
TRAPS		2	D12E, Y20H/D, H22Y, C29F/Y, C30R/S/Y/F, C33G/Y			3	T37I, Y38C, L39F, D42del, C43R/Y/S, P46L, T50M, C52R/F/Y, C55R/S/Y, F60L/S/V, T61I, N65I, L67P, H69fs, C70R/S/G/Y, C73R/W, S74C		
		4	S86P, C88R/Y, R92Q, V95M, C96Y, C98Y, delY103-R104, R104Q, H105P, F112I			6	I170N, V173D		
CAPS		3	C148Y, R168Q, I172T, V198M, C259W, R260W, V262G, L264V, G301D, D303N, E304K, L305P, Q306L, G307V, F309S, E311K, H312P, P315L, G326E, T348M, A352V, L353P, E354D, A374D, M406I, T436A, F443L, N447K, I480F, R488K, E525K, Y563N, G569R, L571F, F573S, T587I, E627G, L632F, M659K, M662T, E688K, E690K, Q703K, S710C						
NLRP12AD		3	D142N, T260M, R284X, D294E, H304Y, P319R, R352C, F402L						
HIDS		2	delEx2, L6fs, H20N/Q	3	delEx3, L29fs, L39P	4	E93fs, Y114fs, I119M	5	DelEx5, V132I, S135L, G140fs, A141fs, A147T, A148T, Y149X, L168fs, G171R
		6	W188X, Q190fs, V203fs/A, N205D, T209A	7	G211A/E R215Q	8	T237S, L246P, V250I	9	L265R, I268T, S272F, R277C/H, P288L, V293M
		10	G309S, T322S, G326R, S329N/R, G336S			11	D366F/S, C367S, G376V, V377I, S378P, H380R, D386N, R388X		
PAPAs		3	R52Q	10	A230T	11	E250Q, E250K, D266N		
DIRA		2	N52KfsX25, Q54X	3	p.Asp72_Ile76del, E77X, C91F	4	Q119X		
MS		4	T180fs	7	A331S, P348L, K387E	9	p.Ser439, Trpfs*15		
		10	L504F	14	E601K, P626S	17	S734L		
EOS BS		4	R334Q/W, E383K, D382E, L469F, W490L, C495Y, H496L, M513T, R587C, T605P, N670K						
DITRA		2	R10X	3	L27P, H32R, K35R	4	N47S, R48W, P76L	5	E94X, R102W, R102Q, S113L, T123R, T123M, G141Mfs*29
CANDLE		1	Q49K	2	T74S, T75M	3	M117V, C135X	5	G201V
CAMPS		2	R69Q	3	G117S	4	E138del, E138A, L156P, D176H, S200N	18	R826W

BS: Behcet's syndrome; CAMPS: CARD14-mediated pustular psoriasis; CANDLE: chronic atypical neutrophilic dermatosis, lipodystrophy and elevated temperature; CAPS: Cryopyrin-associated periodic syndrome; DIRA: interleukin-1 receptor antagonist deficiency; DITRA: IL-36 receptor antagonist deficiency; EOS: Early-onset sarcoidosis; FMF: Familial Mediteranean fever; HIDS: hyperimmunoglobulin D syndrome; MS: Majeed syndrome; MVK: Mevalonate kinase; NLRP12AD: NLRP12-associated syndrome; NOD2: Nucleotide oligomerization domain 2; PAPAs: Pyogenic arthritis, pyoderma gangrenosum and acne syndrome; TRAPS: tumor necrosis factor receptor-associated periodic syndrome

Table 2. Commonly used methods in the genetic diagnosis of hereditary autoinflammatory diseases

Test	Principal of the method	Scope	Advantages	Disadvantages	Reference
Strip-assay	PCR or reverse hybridization only for previously defined variants	Screening of approximately 10-30 known variants	• Inexpensive • Quick (in 1-2 days) • No requirement for too much expertise	• Increased probability of erroneous result • Ability to screen a low number of variants	4
Sanger sequencing	Exon-intron (meanly 500 base pairs) chain termination-based sequencing PCR for desired regions and reading on automated capillary DNA device	Ability to detect all variants in the selected region	• Ability to obtain a result in a mean period of two weeks • Awailability of automated DNA analysis in developed laboratories • A broad scope	• Requirement for expertise in reading and interpretation of the results • Probability of erroneous readings and repetitions • Expensive for large gene regions and multiple genes	5
Next-generation sequencing	Use of pyrosequencing, reverse termination or native nucleotides in synthesis-mediated sequencing principle	Ability to analyse a whole gene and all genes	• Ability to obtain results in 2-10 days depending on the system used • Imaging of all variants at the same time • Low probability of erroneous results given by the individual who performs the test	• Expensiveness • Requirement for special device • Requirement for expertise in bioinformatics and genetics	6

PCR: polymerase chain reaction

in some populations, including the Turkish population, indicate the need for neonatal screening for FMF. This need is more pressing in populations with high rates of consanguineous marriages, such as the Turkish population. Moreover, genetic testing in the diagnosis of FMF is important in terms of preventing injury caused by amiloid-A (AA) amyloidosis, which is the most severe complication of the disease. Previous studies have shown that the risk of developing severe disease phenotype is higher in patients who are homozygous for M694V (10). It was observed that the homozygous state for M694V was a high risk factor for amiloidosis in Armenians, Israelis, and Arabs (11). In a meta-analysis that included 3505 Turkish patients, it was shown that 189 of 400 patients who developed amilosidosis were M694V homozygous (12). Considering that amiloid-A amiloidosis leads to renal injury and multiple organ failure in the long term, if not treated, identifying individuals who are M694V homozygous and initiating treatment or close follow-up even if they are asymptomatic or have moderate symptoms is very important in terms of the course of the disease.

1.2. Periodic syndrome associated with tumor necrosis factor receptor

Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) was first described in 1982 as familial periodic fever (FPF) with autosomal dominant inheritance (13). Periodic syndrome associated with tumor necrosis factor receptor, which commonly occurs in Europeans with Scotch-Irish origin, is also observed in Ashkenazi and Seferad Jews and Israil-end Armenian Arabs (1). Although the disease mostly manifests clinically in childhood (average three years of age), the diagnosis may be made in later childhood or in adulthood. The periodic syndrome associated with tumor necrosis factor receptor gene is *TNFRSF1A*, which is composed of 10 exons and encodes tumor necrosis factor receptor-1 (TNFR1), is a 55-kDa transmembrane glycoprotein. One hundred five of 146 defined variations, which were observed especially on the 2nd, 3rd, 4th, and 6th exons, were associated with the disease (9). Mutations, which show high penetrance, cause disruption in the three-dimensional structure of the TNFR1 protein and inhibit migration of this protein to the cellular surface and attachment to TNF. The protein, which remains inside the cell, leads to the picture of TRAPS by inducing inflammation (14). Disease-onset is earlier and the clinical course is more severe in individuals who carry these mutations. Mutations including R92Q and P46L cause the disease to occur later and have a milder clinical picture (15). In the study conducted by our group, the 2nd, 3rd, and 4th exons in the *TNFRSF1A* gene were sequenced in 50 individuals who were suspected to have TRAPS; heterozygous c.236C>T variations (rs104895219, exon 3, pathogenic mutation, p.Thr79Met-T50M) were de-

fined in two individuals and a heterozygous c.123T>G variation (rs104895271, exon 2, non-pathogenic, p.Asp-41Gul-D12D) was defined in one individual (16).

1.3. Blau syndrome

Blau syndrome is a rare autosomal dominant, autoinflammatory disease that predominantly occurs in Caucasians and is characterized by granulomatous recurrent uveitis, dermatitis, and symmetrical arthritis. Following demonstration of the fact that three missense mutations in the *CARD15/NOD2* gene (R334Q, R334W, and L469F) cause Blau syndrome, different *CARD15/NOD2* mutations have been identified through genotyping in many study groups of different ethnic origins (17). The *CARD15/NOD2* gene encodes the nucleotide oligomerization domain 2 (NOD2) protein, which possesses 1040 amino acids and multiple areas. The nucleotide oligomerization domain 2 is expressed in myelomonocytic, dendritic, and Paneth cells, and has a significant role in the innate immune system. Most of Blau mutations are constituted by mutations that cause changes of the amino acid arginine in the 334th position (R334W or R334Q) (18, 19). In cases when it is clinically difficult to differentiate patients with Blau syndrome from others with inflammatory chronic recurrent arthritis, molecular genetic analysis should be performed for a clear diagnosis. In the analyses performed by our group, the 4th exon of the *NOD2* gene was screened in 27 individuals who were suspected to have Blau syndrome and no pathogenic mutation was found in any of these patients (16).

1.4. Cryopyrin-associated periodic syndromes

Cryopyrin-associated periodic syndromes (CAPS) are a rare, autoinflammatory disease group that generally has autosomal dominant inheritance. CPAS has three clinical subtypes according to the severity of the disease: Familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and chronic infantile neurologic cutaneous articular syndrome/neonatal-onset multisystemic inflammatory disease (CINCA/NOMID). Dominant missense mutations in the *NLRP3* gene found in the chromosome region 1q44 are responsible for most cases of CAPS (20). It is thought that the basic mechanism in the pathogenesis of CAPS includes NLRP3 activation caused by missense mutations and inflammation due to the resultant increase in IL-1 production in monocytes and macrophages. Different activation levels caused by *NLRP3* mutations are thought to be associated with the occurence of three phenotypes with different clinical severities. Similar clinical pictures exhibited by patients who do not carry *NLRP3* mutations support the assumption that other modifying genes or environmental factors may affect the disease phenotype (21). The fact that 13 variations including 3 pathogenic ones were found in the sequencing of 3 exons in the *NLRP3* gene in 55 patients who presented with suspected CAPS in our study supports this opinion (16).

1.5 Hyperimmunoglobulin D syndrome

Hyperimmunoglobulin D syndrome (HIDS) is an autosomal recessive, autoinflammatory disease that occurs in infacy with fever episodes lasting for 3-7 days, recurs every 4-6 weeks, and is mostly observed in Caucasians. The hyperimmunoglobulin D syndrome gene has been mapped to the mevalonate kinase *(MVK)* gene found on the 12th chromosome (22). Mevalonate kinase is involved in the sterol biosynthesis pathway. Although it is known that mutations in this gene lead to a decrease in MVK protein levels, the pathogenesis has yet to be fully elucidated. The differential diagnosis of hyperimmunoglobulin D syndrome from the other autoinflammatory diseases is made through the patient's clinical picture, medical and familial history, and subsequent genetic analysis. However, MVK mutations have been found in only 2% of patients who had a negative test for FMF and at least one of the main clinical characteristics observed in HIDS (23). Therefore, it is important to select patients in whom genetic analyses will be performed for the detection of MVK mutations. In the analyses performed by our group, the 2nd, 3rd, 4th, 6th, and 8-9th exons of the MVK gene were screened in 27 individuals who were suspected of having HIDS, and nine variations with unknown function were detected including two known pathogenic ones and two new variants (16).

1. 6. Methods used in the genetic diagnosis of hereditary autoinflammatory diseases

Although genetic diagnosis is important in these diseases, examination of certain regions of the disease genes or known single mutations in the studies of our group and other studies published so far is mostly not sufficient. In one study, it was reported that no mutation was found with Sanger sequencing in 50% of patients who had systemic autoinflammatory diseases and new variants could also be found together with almost all expected variants when 10 genes that are involved in most of these diseases were screened with next-generation sequencing (24). Sanger sequencing may also fall short due to is inability to detect somatic mosaicism in some patients. Somatic mosaism was found with massive parallel sequencing in six of eight patients who presented with suspected cryopyrin-associated peirodic syndrome and were shown not to

carry *NLRP3* mutations with Sanger sequencing and these individuals were found to carry known/new pathogenic mutations. Next-generation sequencing was performed for the remaining two patients; an *NOD2* mutation was observed in one of these patients and Blau syndrome was diagnosed instead of CAPS in this patient (25). More importantly, screening of single suspected genes including CAPS and TRAPS, TRAPS and HIDS or HIDS, TRAPS, CAPS and FMF with standard sequencing is not efficient in terms of time and cost when patients with multiple suspected diagnoses are referred to genetic diagnosis laboratories. In such cases, it seems to be more beneficial to simultaneously screen autoinflammatory genes that have been identified and are observed commonly using NGS methods.

2. Known susceptibility genes in commonly observed diseases with complex inheritance

Conditions with complex inheritance including juvenile idiopathic arthritis (JIA), systemic lupus erythematosus (SLE), Crohn's disease, and Behçet's syndrome (BS), are among childhood rheumatic diseases. Juvenile idiopathic arthritis is known as chronic rheumatism of childhood and occurs as a result of interaction of genetic and environmental factors. It involves autoimmune and inflammatory characteristics and shows clinical heterogeneity. Although genetic epidemiologic studies have shown that JIA can be inherited, most cases occur sporadically. Different variations have been identified in many different genes in the occurrence of juvenile idiopathic arthritis. Allele variations that have been defined in the human leukocyte antigene (HLA) region and produce different antigenes predominate. Different HLA alleles associated with certain clinical pictures and different JIA subtypes have been found. For example, it was observed that the *DRB1*08- DQA1*0401-DQB1*0402* haplotype increased the tendency to persistent and extended JIA and the *DRB1*01-DQA1*0101-DQB1*0501* haplotype was related with a risk of enthesitis-related arthirits (ERA) and psoriatic arthritis (26). In contrast, the inability to define HLA alleles strongly associated with systemic JIA (sJIA) may suggest that sJIA is directed by antigenes. However, a recent study found that the *DRB1*11* allele and *DRB1*11-DQA1*05-DQB1*03* haplotype were quite strongly associated with sJIA (27). One of the JIA-associated variations identified outside the human leukocyte antigen region is the C1858T T allele and T/T genotype in the *PTPN22* gene (28). In addition, associations of variations in other genes including *MIF, SLC11A6, WISP3*, and *TNF* with JIA have been demonstrated (29, 30).

Systemic lupus erythematosus, which is another condition with complex inheritance, is a chronic, systemic, autoimmune disease that generally involves the skin, bone, kidneys, lungs, and central nervous sytem, with a variable clinical picture. The association rates shown in twin studies and prevalence results in first-degree/second-degree relatives indicated a significant role of genetics in the occurrence of SLE (31, 32). It is thought that polymorphisms in the *FCGR2A, -2B, -3A and -3B* genes encoding Fc gamma receptors, which are known to have a significant role in occurrence of the immune response, may be associated with SLE (33). The R620W variation in the 14th exon on the *PTPN22* gene has also been associated with a risk for SLE as well as with many autoimmune diseases (34). Polymorphysims on the *STAT4* gene have also been shown to be associated with SLE. Among these, the intronic rs7574865 variation is thought to lead to a more severe phenotype. *DRB1*0301* and *DRB1*1501* alleles on the human leukocyte antigen locus have also been observed to be associated with a risk for SLE (35). Additionally, it has been shown that variants in the genes involved in immunologic pathways including *TNFAIP3* may increase the risk for SLE occurence (36). To date, more than 100 variations with a relative risk ranging between 1.15 and 2 have been identified in association with SLE and most cases of SLE are thought to occur as a result of accumulations these factors.

Crohn's disease is the chronic, recurring type of inflammatory bowel disease. Twin studies and familial aggregation findings have shown the role of genetics in the occurrence of Crohn's disease (37). Variations in the *NOD2* gene, which is involved in microbial recognition, are the stongest genetic risk factors defined for Crohn's disease (38). R702W, G908R, and L1007fsinsC are the most common *NOD2* mutations in Crohn's disease. Another variation that has been shown to have a strong relation with the disease is the T300A polymorphism in the *ATG16L1* gene, which is involved in the occurrence of autophagosome (39). Again, variations in the *IRGM* and *LRRK2* genes, which are involved in the regulation of autophagia, have also been associated with Crohn's disease (40). One of the loci that contribute to the risk of Crohn's disease to a significant extent is the HLA region. In a meta-analysis, it was shown that *DRB1*0410* and *DRB1*0103* among class II HLA alleles and *Cw8* and *B21* among class I HLA alleles were HLA variants that showed the strongest relation with Crohn's disease (35). Other *HLA* alleles and variations in genes generally related with lymphocyte activation, survival, and growth including *PTPN22* and *IL23R* were also shown to be related with Crohn's disease (40).

Behçet syndrome (BS) is an autoinflammatory disorder that involves multiple systems and is characterized by mucosal ulceration and neutrophilic inflammation in immune-protected areas including the eye, brain, and synovial joints (41). Although many cases of BS are sporadic, familial aggregation is observed with varing frequencies in different populations (more frequenct in juvenile patients) (42, 43). The strongest genetic factor associated with the risk of Behçet syndrome is the *HLA-B5/B51* allele carrier state, which is observed more frequently in familial cases compared with sporadic BS (44, 45). In a meta-analysis that comprised 4 800 patients with BS and 16 289 controls, the *HLA–B51/B5* frequency was found as 57.2% in patients with BS, and 18.1% in the controls (OR 5.78, 95% CI: [5.00–6.67]) (45). In another study, it was shown that the *HLA-B*5101* variant had a strong relation with BS, and the *MICA-A6* allele was reported to increase predisposition to BS (46). There is also evidence showing an epistatic interaction between *ERAP* and *HLA-B51*, as well as an association between the *STAT4*, *CCR1*, *KLRC4*, and *ERAP1* genes with BS (47). In addition, different studies have shown an association between other genes and loci including *HLA-A26, KIAA1529, CPVL, LOC100129342, UBASH3B, UBAC2, IL10, IL23R-IL12RB2*, and *TNF* and BS risk (48-50).

3. Definition of new genes and diseases in familial cases with an unclear clinical picture

Although the number of rare diseases is not known exactly, the *Mendelian Inheritance in Man* (OMIM) database has reported that there are propably more than 24000 genetic diseases or phenotypes and the number of diseases for which a molecular basis (etiology) has been explained among these is approximately 4 200 (http://www.omim.org/statistics/entry). Considering the mutation occurrence rate and the number of known genes in the human genome, the number of genetic diseases is expected to increase to around 28000. It is known that approximately 7000 of the predicted genetic diseases are observed very rarely.

The genes of diseases can be found or new diseases can be identified with disease gene research studies in patients who present with autoinflammatory signs, but who do not fully comply with known diseases, and have more than one affected individual in the family. In all families evaluated in this context, the hereditary model is identified primarily (autosomal or gonosomal, reccessive or dominant), and the genomic regions responsible for the disease are investigated using linkage analysis methods with appropriate genotyping tools. In the candidate gene regions found as a result of linkage analyses, candidate genes that comply with the phenotype to the greatest extent are investigated. Homozygosity mapping is one of the most efficient methods in cases where the disease is inherited autosomal recessively because consanguineous marriages are common in our country. This approach is based on the fact that the possibility of a homozygous carrier state for disease-related loci is very high in these patients. Another efficient method for narrowing candidate gene regions is "exom sequencing," which involves sequencing of only protein-encoding regions of the genome. Thus, it is possible to determine genetic mutations that are responsible for disease. Subsequently, cadidate genes and mutations should be confirmed using Sanger sequencing and the frequencies of inheritance in the healthy population should be investigated. As a result of these studies, it will be possible to open diagnostic and therapeutic paths for these conditions.

In conclusion, many new diseases have been identified with the discovery of new genes and mutations since the time when hereditary autoinflammatory diseases were first described. Despite information related with different clinical associations in these diseases, and exclusion criteria directed to a diagnosis of suspected autoinflammatory disease, a definite diagnosis mostly requires molecular genetic analyses. Interpretation of genetic results requires a certain level of expertise and is error-prone. The potential effects of genetic variation types found in patients (high or low penetrance mutations, polymorphisms) on the disease pathology should be explained. As explained above, positive genetic evidence cannot be found in a certain percentage of patients who have very typical clinical pictures. Therefore, it is important that consultant centers work in collobroration with experts of genetics. In addition, diseases in the MAD group are significant in terms of the number of individuals affected in the population because the incidence may be very high, though these diseases are included in the class of "rare diseases." Specifying the genetic changes that cause disease in this group of familial diseases will enlighten both the mechanism of diseases and development of potential therapeutic probabilities.

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