

Risk of autoimmune diseases in patients with RASopathies: systematic study of humoral and cellular immunity

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Research

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Abstract

Abnormalities of the immune system are rarely reported in patients affected by RASopathies. Aim of the current study was to investigate the prevalence of immune system dysfunction in a cohort of patients affected by RASopathies.

Study Design. A cohort of 69 patients was enrolled: 60 at the Federico II University, Naples, 7 at University Magna Graecia of Catanzaro, 2 at “ScuolaMedicaSalernitana”, Salerno. An age- and sex-matched control group was also enrolled. Autoimmune disorders were investigated in the study cohort according to international consensus criteria. Immune framework was also evaluated by immunoglobulin levels, CD3, CD4, CD8, CD19, CD56 lymphocyte subpopulations, autoantibodies levels and panel of inflammatory molecules, in both patients and controls.

Results. Frequent upper respiratory tract infections were recorded in 2 patients; pneumonia, psoriasis and alopecia in single patients. Low IgA levels were detected in 8/44 patients (18.18%), low CD8 T cells in 13/35 patients (37.14%). Anti-tg and anti-TPO antibodies were detected in 3/24 patients (12.5%), anti-rTSH in 2 cases (8.33%), all in euthyroidism. Serum IgA and CD8 levels were significantly lower in patients than in controls ($p = 0.00685$; $p = 0.000656$ respectively). All tested patients showed increased inflammatory molecules compared to controls. These findings may anticipate the detection of overt autoimmune disease.

Conclusions. Patients affected by RASopathies are at risk to develop autoimmune disorders. Routine screening for autoimmunity is recommended in patients with RASopathy.

Introduction

RASopathies are a clinically defined group of medical genetic syndromes caused by germline mutations in the genes that encode components or regulators of the RAS/mitogen activated protein kinase (MAPK) pathway. Taken together, the RASopathies represent one of the most prevalent group of congenital malformation syndromes affecting approximately 1 in 1,000 individuals[1].

The RAS/MAPK pathway is a ubiquitous, highly conserved, intracellular signaling pathway that is critical in the cell cycle regulation, differentiation, growth, apoptosis and cell senescence. So, mutations that affect its function can have a profound deleterious effect on development[1]. Abnormalities in RAS expression, activation, and signaling pathways appear to play also an important role in the regulation of the inflammatory response and in autoimmune mechanisms [2-5].

RASopathies group include: Noonan syndrome (NS) caused by mutations in *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *NRAS* and *CBL*; NS-like with loose anagen hair (NSLAH) due to germline mutations of *SHOC2*[6] or more rarely, *PPP1CB*[7]; NS with multiple lentiginos (NSML) caused by specific mutations of *PTPN11*[8], although other rare mutations have been reported[9]; Costello syndrome (CS) caused by activating mutations in *HRAS*; cardio-facio-cutaneous syndrome (CFC) caused by gain of function mutations in *BRAF* and *MAP2K1* or *MAP2K2*[1]. Heterozygous missense mutations in *MAP2K1* (*MEK1*) and *MAP2K2* (*MEK2*) are present in approximately 25% of CFC individuals[10]. Mutations *RIT1* have been identified in 17 of 180 patients (9%) with Noonan syndrome or a related condition but with no detectable mutations in known Noonan-related genes[11]. *LZTR1* may be responsible of a rare percentage of NS cases[1].

RASopathies are multisystemic disorders with a unique phenotype, but they share many overlapping characteristics, including craniofacial dysmorphism, cardiac malformations, cutaneous, musculoskeletal, and ocular abnormalities, neurocognitive impairment; hypotonia and an increased cancer risk [12].

ARAS-associated autoimmune leukoproliferative disorder (RALD) has been described, characterized by a non-malignant clinical picture, partly overlapping to that of autoimmune lymphoproliferative syndrome (ALPS), represented by lymphadenopathy, splenomegaly, increased circulating B lymphocytes, hypergammaglobulinemia and autoimmunity. Unlike ALPS, RALDs do not generally show increased values of circulating double negative T lymphocytes, increased values of vitamin B12 and mutation of FAS, FASL or CASP10[13].

Autoimmune diseases have rarely been described in NS. Case reports of patients with NS and autoimmune diseases such as systemic lupus erythematosus, celiac disease, Hashimoto thyroiditis[14] and chronic idiopathic thrombocytopenic purpura have been described[15]. Few cases of NS associated with autoimmune hepatitis have also been reported[16]. A cohort of patients with RASopathies including 42 patients showed a high frequency of positivity of autoantibody titles, in the presence or absence of associated clinical manifestations[17].

The aim of the present study was to investigate the prevalence of immune system dysfunction and autoimmune disorders in a cohort of patients affected by RASopathies.

Patients And Methods

A cohort of 69 patients (43 males, 26 female) was enrolled in the study: 60 at the Pediatric Genetic Section of the University Federico II of Naples, 7 at the Department of Clinical and Experimental Medicine of the University Magna Graecia of Catanzaro and two at Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", Salerno (Italy).

Clinical diagnosis were: 57 NS, 5 CFC, 3 LS, 4 NF1/NS. The mean age at the moment of the enrolment was 8.72 years, (ranges 0 to 26 years). A control group was also enrolled including 50 subjects (30 males, 20 females) mean age 8.7 years (ranges 0-26).

Inclusion criteria was represented by diagnosis of Rasopathies, based upon clinical features and confirmed by molecular analysis performed on DNA extracted from circulating leucocytes. The patients enrolled presented the following genetic mutations distribution: 56.52% *PTPN11*, 13.04% *SOS1*, 11.59% *BRAF*, 5.8% *RIT1*, 4.35% *LZTR1*, 2.9% *RAF1*, 1.45% *KRAS*, 1.45% *MAP2K2*, 1.45% *MEK1*.

This is a retrospective study: all patients underwent anamnestic recall, clinical examination, including auxological parameters. Clinical findings suggestive for infectious disease or auto-immune disorder were recorded including: upper and lower airway infections, otitis, skin infection and/or presence of arthralgia, arthritis, purpura. For all the categories, the type of defects and the frequency of the individual anomalies were analyzed. All autoimmune disorders were excluded or diagnosed in the study cohort according to international consensus criteria [18-24]. In all patients complete blood count, determination of C-reactive protein and thyroid profile were performed.

In a group of 44/69 patients and 30/50 controls quantitative analysis of immunoglobulin levels (IgA, IgG, IgM, IgE), was performed and interpreted according to the normal range (\pm 2SD) proposed by Ugazio et al [25].

In a group of 35/69 patients and 50/50 controls CD3, CD4, CD8, CD19, CD56 lymphocyte subpopulations was performed by FACS and the normal range was considered according to the scheme provided by **Dallavilla et al**[26].

Patients sample (24/69) were screened for antinuclear antibodies (ANA) by ELISA. Dilutions 1:320 were defined as positive. Anti Tg, anti-TPO, anti r-TSH anti and LKM1 antibodies were assayed by ELISA. ENA and anti-dsDNA were measured by chemiluminescence. Rheumatoid factor (RF), anti-double-stranded DNA (anti-dsDNA), Anti-smooth muscle antibodies, anticardiolipin, Lupus anticoagulant, Anti-neutrophil cytoplasm antibody (ANCA), anti Tgasi, anti-beta 2 glycoprotein 1, glutamic acid anti-decarboxylase (GAD), anti-insulin (IAA), anti-tyrosine phosphatase (IA-2A) and anti-zinc transporter 8 were also detected. The serum levels of the C3 and C4 complement components were determined.

In a group of 10/69 patients and 10/50 controls screening of a panel of inflammatory molecules was performed including PDGF, IL-1b, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, Eotaxin, FGF basic, G-CSF, GM-CSF, IFN-g, IP-10, MCP-1(MCAF), MIP-1a, MIP-1b, RANTES, TNF-a, VEGF.

Statistical Analysis

Each numerical variable is expressed as mean +/- SD. Statistical analysis was performed using SPSS package.

Differences in the lymphocytes, autoantibodies and inflammatory molecules levels between patients and controls were analyzed using the *t-test* for unpaired data corrected for Fisher exact test. To investigate the presence of an association between severity of phenotype and either DNA mutation or specific gene involved, χ^2 test was performed. To investigate the presence of a correlation between the severity of phenotype and specific gene involved, spearman correlation study and association study were performed.

A P value < 0.05 was considered to be significant in all instances.

Results

Clinical parameters

All patients underwent anamnestic recall, clinical examination, including auxological parameters, clinical findings suggestive for infectious disease or auto-immune disorder. Auxological parameters reveal short stature for specific growth chart in 21/69 patients.

Upper respiratory tract infections were recorded in 2 patients (2/69; 2.89%) and pneumonia in 1 patient (1/69, 1.45%).

In no case an autoimmune disorder was diagnosed, except for one case of psoriasis in a patient (1/69, 1.45%) with SOS1 mutation. Alopecia and leukemia were detected in a single patient (**Table 1**)

Biochemical parameters

Altered values of Ig levels were recorded in 10/44 patients, 22.72%. Low IgA levels were found in 8/44 patients, 18.18% (**Supplementary Table S1**).

Decreased values of CD3 and/or CD8 were recorded in 14/35 patients (40%). CD19 was reduced in 4 cases (4/35, 11.43%) (**Supplementary Table S2**).

One of the patients with PTPN11 mutation and a reduction in the TCD8 value also had a reduction in IgA and IgG.

A total of 6 patients out of 24 (25%) presented with at least one autoantibody described below. Four presented with the concomitant occurrence of two autoantibodies. Anti-tg were detected in 4/24 patients (16.6%), 3 of these (12.5%) also showed anti-TPO antibodies. Anti r-TSH in 2/24 patients (8.33%), all in euthyroidism. The presence of Anti-LKM1 was detected in one patient (4.16%) who showed anti r-TSH also.

Screening of a panel of inflammatory molecules revealed a significant increase of IL(Interleukin)-1ra, IL-2, IL-4, IL-6, IL-7, IL-10, IL-15, Eotaxin, G-CSF and IP-10, in all patients tested. **(Table 2)**.

Statistical Analysis

Immunoglobulin data, lymphocyte classes and inflammatory molecules were analysed in patients and controls. IgA levels were lower in patients than in controls (p 0,00685) **(Table 3a)**. Patients showed lower CD8 than controls (p 0,000656) **(Table 3b)**.

Inflammatory molecules were significantly higher in patients than in controls: IL1-ra (0,002646), IL-2 (p 0,027678), IL-4 (p 0,017983), IL-6 (p 0,033026). IL-7 (p 0,012856). IL-10 (p 0,014939). IL-15 (p 0,01665). Eotaxin (p 0,000724). G-CSF (p 0,014625). IP-10 (0,029932) **(Table 2)**.

Correlation analysis showed that BRAF patients have higher prevalence of CD19 deficiency (R -0.36; p 0.049).

Discussion

Abnormalities of the immune system or autoimmune diseases are rarely reported in patients affected by RASopathies. The current prospective study analysed immune system dysfunction and autoimmune biological markers in a cohort of 69 patients and 50 controls.

The results of the current study showed lower IgA levels in patients than in controls (p 0,00685). The worldwide prevalence of selective IgA deficiency depends on the ethnic background and it is most prevalent in Caucasians (1:600) [27]. Most individuals are asymptomatic, but the defect may be associated with recurrent respiratory and gastrointestinal tract infections/disorders, autoimmunity and allergies [28]. There is only one case reported in the literature of a 32-month-old female with Noonan and hypogammaglobulinemia, placed on antibiotic prophylaxis [29]. Four of our patients with IgA deficiency also had IgG and/or IgM deficiency. Upper respiratory tract infections were recorded in 2 patients and pneumonia in 1 only.

Recently, a cohort of 42 patients with RASopathies was evaluated for autoimmune status. Autoimmune antibodies were observed in 52% of the patients. Remarkably, three (7%) of the patients had specific gastrointestinal and liver autoantibodies without clinical findings. Six patients (14%) fulfilled the clinical criteria for autoimmune diseases [systemic lupus erythematosus, polyendocrinopathy (autoimmune thyroiditis and celiac disease), primary antiphospholipid syndrome, autoimmune hepatitis, vitiligo, and autoimmune thyroiditis] [17]. Other cases of autoimmune diseases are reported anecdotally in patients with Rasopathies [2, 30-33].

Although clinical findings suggestive for autoimmune disease were detected in only one patient, biochemical parameters showed specific alterations.

Our study has highlighted the frequent finding of positivity for thyroid autoantibodies (25%), all in conditions of euthyroidism, as already reported [34-35]. Autoimmune diseases, which are significantly increasing in the world population, currently have an estimated prevalence of around 3% in childhood and adolescence: frequent clinical pictures (constituting about 75% of total cases) include autoimmune thyroid disease (TAI), celiac disease (CD) and juvenile idiopathic arthritis (JIA). The most common form of TAI, Hashimoto's thyroiditis, shows a frequency of 1.3% in the pediatric population and mainly affects women [36], but it reaches 3% if the prevalence of antithyroid antibodies is evaluated which are also found more frequently in girls with goiter and after 12 years of age [37]. However, in recent years, numerous prospective studies have demonstrated that many autoantibodies can be detected in the serum of asymptomatic or paucisymptomatic individuals who later develop an autoimmune disease. These antibodies can therefore precede the clinical symptoms of the disease by years, and could in principle be used for diagnostic and prognostic purposes, including screening studies [38].

Reduced CD8+ T-cells levels were also demonstrated in our patients(**Figure 1**).

As known, the Ras/MAPK cascade is involved in 'immunological tolerance', that is the ability to distinguish between self and non-self and excessive activation of MAPKs is associated with autoimmune diseases. It has been proved that a small increase in ligand affinity for the T-cell antigen receptor leads to a marked change in the activation and subcellular localization of Ras and mitogen-activated protein kinase (MAPK) signalling intermediates and the induction of negative selection [39]. Moreover, Ras/MAPK signalling is also implicated in peripheral tolerance to prevent autoimmune destruction by self-reactive T cells that escape thymic deletion. In particular, Erk MAPK pathway plays a critical role in CD8 T cell activation, proliferation, and survival [40]. Peripheral deletion of CD8 T cells requires p38 mitogen activated protein kinase in cross-presenting dendritic cells and p38 inhibition could rescue CD8 T cells from Bim-dependent apoptosis [41].Activation of the MAPK kinase p38 signaling pathway had previously been reported to selectively induce apoptosis in CD8 T cells in vivo[42]. Huang et al. demonstrated that, during immune tolerance, p38 α is constitutively activated in cross-presenting Dendritic Cells (DCs) from the mesenteric lymph node, leading to the generation of induced Tregs and inhibition of Th1 T cells through a TGF β dependent mechanism. Consequently, loss of p38 α in DCs prevented induction of oral tolerance in vivo[43].

On the basis of these data it might be suggested that impairment of RAS-MAPK pathway alters CD8 production and function causing intolerance and cross reactivity.

In our study, CD8 deficiency is probably the result of disease-causing mutations enhancing signal flow through RAS/MAPK pathway. Although the role of CD8+ T cells is not as well established, it is known that CD8+ T cells contribute to the induction, progression, pathogenesis and protection from many autoimmune diseases [44]. A recently systematic review, highlighted the potential role of CD8+ T cells in different autoimmune diseases and it has been demonstrated that CD8+T cells are decreased in the peripheral blood of patients with Grave's disease and upregulated in others autoimmune disease according to epigenetic mechanisms which participate in the activation, differentiation, and development of CD8+T cells[45]. Although some studies have not found CD8+ T-cell deficiency in patients with autoimmune diseases or have attributed the deficiency to hormonal factors, CD8+ T-cell deficiency would appear to be a general feature of human chronic autoimmune diseases attributed to sequestration of CD8+T cells in the target organ. CD8+ T-cell deficiency also occurs in healthy blood relatives of patients with these diseases. It is proposed that this deficiency is genetically determined and underlies the development of chronic autoimmune diseases [46].

We hypothesized that reduced CD8+ T-cells levels demonstrated in our patients as a consequence of the alteration RAS/MAPK pathway might predispose to autoimmune disorders patients affected by RASopathies and therefore could be the first detectable sign of possible emergence of autoimmune disease.

The increase of inflammatory molecules levels, in our patients, might instead suggest the presence of a state of chronic low-grade inflammation. On the other hand, cytokines including proinflammatory cytokines (IL-1, TNF α , IFN, IL-2, IL-6, IL-12) and consequently anti-inflammatory cytokines (IL-10, IL-11, IL-13, IL-1ra) are important players in the pathogenesis of autoimmune disease through multiple ways, such as regulating inflammation and angiogenesis [47-48].

For example, our patients showed high levels of IL-4 and it is well known the B-cell stimulatory and Th2 promoting properties of IL-4 in the development of autoantibodies and autoantibody mediated diseases [49]. Even more important, IL-6 is a critical cytokine that mediates numerous inflammatory and immunomodulatory pathways. In this regard, dysregulated and persistent IL-6 production results in severe inflammatory and autoimmune disorders [50]. There are also in support for susceptibility to autoimmune phenomena, increased of IL-7 that abrogates suppressive activity of human

regulatory T cells and allows expansion of alloreactive and autoreactive T cells [51] and increased of IL-15 implicated in the pathogenesis of several immune diseases [52]. Interestingly, IP-10 is also significantly higher in patients than in controls. IP-10 and its receptor, CXCR3, appear to contribute to the pathogenesis of many autoimmune diseases and high levels of circulating IP-10, have been shown in patients with autoimmune thyroiditis also relating to a stronger and more aggressive inflammatory response in the thyroid [53-54].

Likely, as a counter-regulatory action, we demonstrated IL-2 and IL-10 increased, the first performs an essential function in the control immune responses and maintain self-tolerance [55] and the second is another key mediator of the anti-inflammatory response [56] (**Saraiva et al 2020**). It might be suggested that the increase of inflammatory molecules levels with a state of chronic low-grade inflammation represents the underlying pathological mechanism driving immune and metabolic pathways involved in autoimmune diseases.

In conclusion, in light of our data we can underline that the altered regulation of RAS/MAPK pathway that characterizes the cohort of patients could be responsible of the altered function of the immune system. In particular, the results suggested a major tendency to autoimmune phenomena than to an immunodeficiency as demonstrated by the finding of circulating autoantibodies, low levels of CD8 T cells and high levels of inflammatory cytokines. So, these evidences may be the first markers of the possible evolution in overt autoimmune disease.

Limits of study

A limitation of our study is the lack of a comprehensive study in the literature that has determined the exact frequency of the autoimmune markers or autoimmune diseases in the general population which allows comparison with RASopathy patients. In addition, we have not considered a control group for the detection of autoantibodies.

Moreover, the average age of our patients is relatively low, which probably limits the diagnosis of autoimmune disorders that have a later onset.

Conclusion

Patients with RASopathies show IgA deficiency, low TCD8 lymphocytes count and high inflammatory molecules levels. The detection of autoantibodies may anticipate the detection of overt autoimmune disease.

A comprehensive clinical and biochemical assessment should be carried out both at diagnosis and during the follow-up. We suggest the importance to include the dosage of serum immunoglobulins, cytokines and lymphocyte classes among the annual screening tests performed in this group of patients. A correct endocrinological follow-up with thyroid profile is worthwhile, considering the high prevalence of positivity for autoantibodies.

In order to recommend routine screening for autoimmunity in patients with asymptomatic RASopathy, continuous monitoring will be required for possible emergence of autoimmune disease. Other studies are also needed to confirm our data.

Abbreviations

MAPK: mitogen activated protein kinase

NS: Noonan syndrome

NSLAH: NS-like with loose anagen hair

CS: Costello syndrome

CFC: cardio-facio-cutaneous syndrome

RALD: RAS-associated autoimmune leukoproliferative disorder

ALPS: autoimmune lymphoproliferative syndrome

NF1: neurofibromatosis type 1

Declarations

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Authors' contributions

M.A. Siano and D. Melis designed and directed the study, and wrote the manuscript. V. Marchetti, S. Pagano, F. Di Candia, M. Alessio, D. De Brasi, S. Sestito, D. Concolino, were in charge of the patients clinical monitoring and collected clinical literature.

De Luca A and Pinna V performed molecular investigations. V. D'Esposito, S. Cabaro, G. Perruolo and P. Formisano performed screening of a panel of inflammatory molecules.

Tartaglia M and P. Strisciuglio critically reviewed the manuscript. D. Melis encouraged the study progress and gave substantial cultural contribution. All authors discussed the results and contributed to the final manuscript.

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Availability of data and materials

Data are available by request

Ethics approval and consent to participate

All procedures followed were in accordance with the ethical standards of the responsible Institutional Committee on Human Experimentation and with the Helsinki Declaration of 1975 (revised in 2000). The study was approved by the Ethics Committee of the University Hospital of Salerno

Consent for publication

Informed consent was written by the parents of patients to participate in this study.

Competing interests

The authors declare that they have no competing interests.

References

1. Tidyman WE, Rauen KA. Expansion of the RASopathies. *Curr Genet Med Rep*. 2016;4(3):57–64. doi:10.1007/s40142-016-0100-7
2. Lopez-Rangel E, Malleson PN, Lirenman DS, Roa B, Wiszniewska J, Lewis ME. 2005. Systemic Lupus Erythematosus and other autoimmune disorders in children with Noonan Syndrome. *Am J MedGenet Part A* 139A:239–242.
3. Mustelin T. 2006. Are other protein tyrosine phosphatases than PTPN22 associated with autoimmunity? *SeminImmunol* 18:254–260.
4. Stone JC. 2006. Regulation of Ras in lymphocytes: Get a GRP. *BiochemSoc Trans* 34:858–861.
5. Vang T, Miletic AV, Bottini N, Mustelin T. 2007. Protein tyrosine phosphatase in human autoimmunity. *Autoimmunity* 40:453–461.
6. Cordeddu V, Di Schiavi E, Pennacchio LA, Ma'ayan A, Sarkozy A, Fodale V, et al. Mutation of SHOC2 promotes aberrant protein N-myristoylation and causes Noonan-like syndrome with loose anagen hair. *Nat Genet* 2009;41:1022–6.
7. Gripp KW, Aldinger KA, Bennett JT, Baker L, Tusi J, Powell-Hamilton N, et al. A novel rasopathy caused by recurrent de novo missense mutations in PPP1CB closely resembles Noonan syndrome with loose anagen hair. *Am J Med Genet Part A* 2016;170:2237–47
8. Tartaglia M, Martinelli S, Stella L, Bocchinfuso G, Flex E, Cordeddu V et al. Diversity and functional consequences of germline and somatic PTPN11 mutations in human disease. *Am J Hum Genet*. 2006;78:279–90.
9. Nishi E, Mizuno S, Nanjo Y, Niihori T, Fukushima Y, Matsubara Y, et al. A novel heterozygous MAP2K1 mutation in a patient with Noonan syndrome with multiple lentigines. *Am J Med Genet Part A* 2015;167A:407–11.

10. Tidyman, WE.; Rauen, KA. Molecular cause of cardio-facio-cutaneous syndrome. Noonan Syndrome and Related Disorders: A Matter of Deregulated RasSignaling. In: Zenker, M., editor. *Monogr Hum Genet.* 17. Basel, Switz: Karger; 2009. p. 73-82.
11. Aoki Y, Niihori T, Banjo T, Okamoto N, Mizuno S, Kurosawa K, et al. Gain-of-function mutations in RIT1 cause Noonan syndrome, a RAS/MAPK pathway syndrome. *Am J Hum Genet* 2013; 93:173–80
12. Rauen KA. The RASopathies. *Annu Rev Genomics Hum Genet.* 2013;14:355–369. doi:10.1146/annurev-genom-091212-153523
13. Calvo KR, Price S, Braylan RC, Oliveira JB, Lenardo M, Fleisher TA, Rao VK. JMML and RALD (Ras-associated autoimmune leukoproliferative disorder): common genetic etiology yet clinically distinct entities. *Blood.* 2015 Apr 30;125(18):2753-8. doi: 10.1182/blood-2014-11-567917. Epub 2015 Feb 17. Review.
14. Amoroso A, Garzia P, Vadacca M, et al. The unusual association of three autoimmune diseases in a patient with Noonan syndrome. *J Adolesc Health.* 2003;32(1):94–97. doi:10.1016/s1054-139x(02)00364-6
15. Flick JT, Singh AK, Kizer J, Lazarchick J. Platelet dysfunction in Noonan's syndrome. A case with a platelet cyclooxygenase-like deficiency and chronic idiopathic thrombocytopenic purpura. *Am J ClinPathol.* 1991;95(5):739–742. doi:10.1093/ajcp/95.5.739
16. Loddo I, Romano C, Cutrupi MC, et al. Autoimmune liver disease in Noonan Syndrome. *Eur J Med Genet.* 2015;58(3):188–190. doi:10.1016/j.ejmg.2014.12.013
17. Quaió CR, Carvalho JF, da Silva CA, Bueno C, Brasil AS, Pereira AC, Jorge AA, Malaquias AC, Kim CA, Bertola DR. Autoimmune disease and multiple autoantibodies in 42 patients with RASopathies. *Am J Med Genet A.* 2012 May;158A(5):1077-82. doi: 10.1002/ajmg.a.35290. Epub 2012 Apr 9.
18. Hochberg MC. 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus *Arthritis Rheum* Sep 40:1725
19. Alvarez F, Berg PA, Bianchi FB, Burroughs AK, Cancado EL, Chapman RW, Cooksley WGE, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston ALWF, Fainboim L, Heathcote J, Homberg JC, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RNM, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Buschenfelde KH, Mieli-Vergani G, Nakanuma Y, € Nishioka M, Penner E, Porta G, Portmann BC, Reed WD, Rodes J, Schalm SW, Scheuer PJ, Schrupf E, Seki T, Toda G, Tsuji T, Tygstrup N, Vergani D, Zeniyaet M. 1999. International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 31:929–938
20. Franklyn JA. Hypothyroidism. *Medicine* 2005. 33:27–29
21. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, DE Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. 2006. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J ThrombHaemost* 4:295–306.
22. Sehgal VN, Srivastava G. 2007. Vitiligo: Compendium of clinicoepidemiological features. *Indian J DermatolVenereolLeprol* 73:149–156.
23. De Block CE, De Leeuw IH, Van Gaal LF. 2008. Autoimmune gastritis in type 1 diabetes: A clinically oriented review. *J ClinEndocrinolMetab* 93:363–371.
24. Catassi C, Fasano A. 2010. Celiac disease diagnosis: Simple rules are better than complicated algorithms. *Am J Med* 123:691–693
25. Ugazio AG et al Il bambino immunodepresso: perché lo è e come va difeso. CEA, 1995

26. La linfopenia nel bambino. Dallavilla, R. Badolato. *Medico e Bambino* 2015;34:239-246.
27. Primary Immunodeficiency Diseases. Report of a WHO Scientific Group WHO. *ClinExpImmunol.* 1997;159:6236–41
28. Wang N, Shen N, Vyse TJ et al. Selective IgA deficiency in autoimmune diseases. *Mol Med.* 2011;17:1383
29. Hostoffer, R.W. Macleish S. 2005. Noonan's syndrome associated with hypogamma -globulinemia *Journal of Allergy and Clinical Immunology*, Volume 115, Issue 2, S160
30. Martin DM, Gencyuz CF, Petty EM. 2001. Systemic lupus erythematosus in a man with Noonan syndrome. *Am J Med Genet* 102:59–62.
31. Berberich MS, Hall JG. 1976. Noonan syndrome-an unusual family with above average intelligence, a high incidence of cancer and rare type of vasculitis. *Birth Defects Orig Artic Ser* 12:181–186.
32. Alanay Y, Balç S, Ozen S. 2004. Noonan syndrome and systemic lupus erythematosus: Presentation in childhood. *ClinDysmorphol* 13:161–163.
33. Yamashita Y, Kusaga A, Koga Y, Nagamitsu S, Matsuishi T. 2004. Noonan syndrome, moyamoya-like vascular changes, and antiphospholipid syndrome. *PediatrNeurol* 31:364–366.
34. Ves
35. Svensson J, Carlsson A, Ericsson UB, Westphal O, Ivarsson SA. 2003. Noonan's syndrome and autoimmune diseases. *J PediatrEndocrinolMetab* 16:217–218
36. Tozzoli. R. Perini. Malattie autoimmuni nei primi anni di vita: dai sintomi alla diagnosi di laboratorio. *RIMeL / IJLaM* 2007; 3
37. Loviselli A, Velluzzi F, Mossa P, Cambosu MA, Secci G, Atzeni F, Taberlet A, Balestrieri A, Martino E, Grasso L, Songini M, Bottazzo GF, Mariotti S, Sardinianschoolchildrenstudygroup. The Sardinian Autoimmunity Study: 3. Studies on circulating antithyroid antibodies in Sardinian schoolchildren: relationship to goiter prevalence and thyroid function. *Thyroid* 11: 849, 2001.
38. Nicola Bizzaro. Autoantibodies as predictors of disease: The clinical and experimental evidence. *Autoimmunity Reviews* 6 (2007) 325–333
39. Daniels MA, Teixeira E, Gill J, Hausmann B, Roubaty D, Holmberg K et al. Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature.* 2006 Dec 7;444(7120):724-9.
40. D'Souza WN, Chang CF, Fischer AM, Li M, Hedrick SM. The Erk2 MAPK regulates CD8 T cell proliferation and survival. *J Immunol.* 2008 Dec 1;181(11):7617-29.
41. Smith T, Lin X, Mello M, Marquardt K, Cheung J, Lu B, et al. Peripheral Deletion of CD8 T Cells Requires p38 MAPK in Cross-Presenting Dendritic Cells. *J Immunol.* 2017 Oct 15;199(8):2713-2720.
42. Merritt C, Enslin H, Diehl N, Conze D, Davis RJ, Rincón M. Activation of p38 mitogen-activated protein kinase in vivo selectively induces apoptosis of CD8(+) but not CD4(+) T cells. *Mol Cell Biol.* 2000 Feb;20(3):936-46.
43. Huang G, Wang Y, Chi H. Control of T cell fates and immune tolerance by p38 α signaling in mucosal CD103+ dendritic cells. *J Immunol.* 2013 Jul 15;191(2):650-9. doi: 10.4049/jimmunol.1300398. Epub 2013 Jun 10.
44. Gravano DM, Hoyer KK. Promotion and prevention of autoimmune disease by CD8+ T cells. *J Autoimmun.* 2013 Sep;45:68-79.
45. Deng Q, Luo Y, Chang C, Wu H, Ding Y, Xiao R. The Emerging Epigenetic Role of CD8+T Cells in Autoimmune Diseases: A Systematic Review. *Front Immunol.* 2019 Apr 18;10:856.

46. Pender MP. CD8+ T-Cell Deficiency, Epstein-Barr Virus Infection, Vitamin D Deficiency, and Steps to Autoimmunity: A Unifying Hypothesis. *Autoimmune Dis.* 2012;2012:189096
47. Guan Q, Zhang J. Recent Advances: The Imbalance of Cytokines in the Pathogenesis of Inflammatory Bowel Disease. *Mediators Inflamm.* 2017;2017:4810258.
48. Andreakos ET, Foxwell BM, Brennan FM, Maini RN, Feldmann M. Cytokines and anti-cytokine biologicals in autoimmunity: present and future. *Cytokine Growth Factor Rev.* 2002 Aug-Oct;13(4-5):299-313.
49. Singh RR. IL-4 and many roads to lupuslike autoimmunity. *ClinImmunol.* 2003 Aug;108(2):73-9.
50. Jordan SC, Choi J, Kim I, Wu G, Toyoda M, Shin B, Vo A. Interleukin-6, A Cytokine Critical to Mediation of Inflammation, Autoimmunity and Allograft Rejection: Therapeutic Implications of IL-6 Receptor Blockade. *Transplantation.* 2017 Jan;101(1):32-44
51. Heninger AK, Theil A, Wilhelm C, Petzold C, Huebel N, Kretschmer K, et al. IL-7 abrogates suppressive activity of human CD4+CD25+FOXP3+ regulatory T cells and allows expansion of alloreactive and autoreactive T cells. *J Immunol.* 2012 Dec 15;189(12):5649-58.
52. Allard-Chamard H, Mishra HK, Nandi M, Mayhue M, Menendez A, Ilangumaran S, Ramanathan S. Interleukin-15 in autoimmunity. *Cytokine.* 2020 Dec;136:155258.
53. Antonelli A, Fallahi P, Rotondi M, Ferrari SM, Romagnani P, Grosso M, et al. Increased serum CXCL10 in Graves' disease or autoimmune thyroiditis is not associated with hyper- or hypothyroidism per se, but is specifically sustained by the autoimmune, inflammatory process. *Eur J Endocrinol.* 2006 May;154(5):651-8.
54. Ruffilli I, Ferrari SM, Colaci M, Ferri C, Fallahi P, Antonelli A. IP-10 in autoimmune thyroiditis. *HormMetab Res.* 2014 Aug;46(9):597-602.
55. Abbas AK, Trotta E, R Simeonov D, Marson A, Bluestone JA. Revisiting IL-2: Biology and therapeutic prospects. *SciImmunol.* 2018 Jul 6;3(25):eaat1482.
56. Saraiva M, Vieira P, O'Garra A. Biology and therapeutic potential of interleukin-10. *J Exp Med.* 2020 Jan 6;217(1):e20190418.
57. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, Wara DW, Douglas SD, Luzuriaga K, McFarland EJ, Yogev R, Rathore MH, Levy W, Graham BL, Spector SA; Pediatric AIDS Clinical Trials Group. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy ClinImmunol.* 2003 Nov;112(5):973-80

Tables

Table 1. Clinical and laboratory features of patients cohort according to genetic mutation									
Gene	PTPN11 (N=37)	SOS1 (N=9)	RAF1 (N=2)	BRAF (N=8)	LZTR1 (N=3)	RIT1 (N=4)	KRAS (N=1)	MAP2K2 (N=1)	MEK1 (N=1)
Infection in regions including the middle ear and upper airway tract	1	1	-	-	-	-	-	-	-
Pneumonia	1	-	-	-	-	-	-	-	-
DecreasedIgA	4	1	-	2	-	1	-	-	-
DecreasedIgG	1	1	-	1	-	-	-	-	-
DecreasedIgM	1	1	-	1	-	1	-	-	-
< CD3	1	-	-	-	-	-	-	-	-
< CD8	2	2	-	1	-	1	-	-	-
Anti Tg	1	-	-	1	1	1	-	-	-
Anti-TPO	1	-	-	1	1	-	-	-	-
Anti R-TSH	2	-	-	-	-	-	-	-	-
Anti-LKM1	1	-	-	-	-	-	-	-	-
ENA /Anti-dsDNA	-	-	-	-	-	-	-	-	-
Autoimmune diseases	-	1 Psoriasis	-	-	-	-	-	-	-

Table 2. Mean of cytokine values in patients and controls			
	Patients (N=10)	Controls (N=10)	p
	Mean ± DS	Mean ± DS	
IL1-ra	840,1±278,9	426,4±251,3	0,002646
IL-2	18,8±6,63	13,72±1,32	0,027678
IL4	6,1±2,90	3,715±0,54	0,017983
IL6	13,41±7,19	7,998±1,799	0,033026
IL-7	46,62±9,37	37,96±3,24	0,012856
IL-10	14,97±4,095	10,89±2,49	0,014939
IL-15	439,42±112,26	324,05±80,61	0,01665
Eotaxin	121,43±32,94	71,757±20,16	0,000724
G-CSF	371,69±221,266	177,45±52,57	0,014625
IP-10	798,15±247,03	575,96±166,79	0,029932

Table 3a. Mean of immunoglobulins values in patients and controls			
	Patients (N=44)	Controls (N=30)	p
	Mean ± DS	Mean ± DS	
IgA	96,35294 ± 49,40044	140,25 ± 34,19762	0,00685
IgG	948 ± 217,1421	866,2667 ± 324,5688	0,219003
IgM	103,027 ± 43,5993	135,4667 ± 32,5727	0,012328

Table 3b. Main lymphocyte subpopulations			
	Patients (N=35)	Controls (N=50)	p
	Mean ± DS	Mean ± DS	
CD3	1887±1279.5	1924,74±558.7	0,854889
CD4	1276,18±1072,57	1026,16± 295,53	0,120953918
CD8	502,84±281.1	707,4±232.7	0,000656
CD19	536,76±412,71	403,82±301,49	0,093913361
CD56	328,57± 311,12	323,98± 129,34	0,92731834

Figures

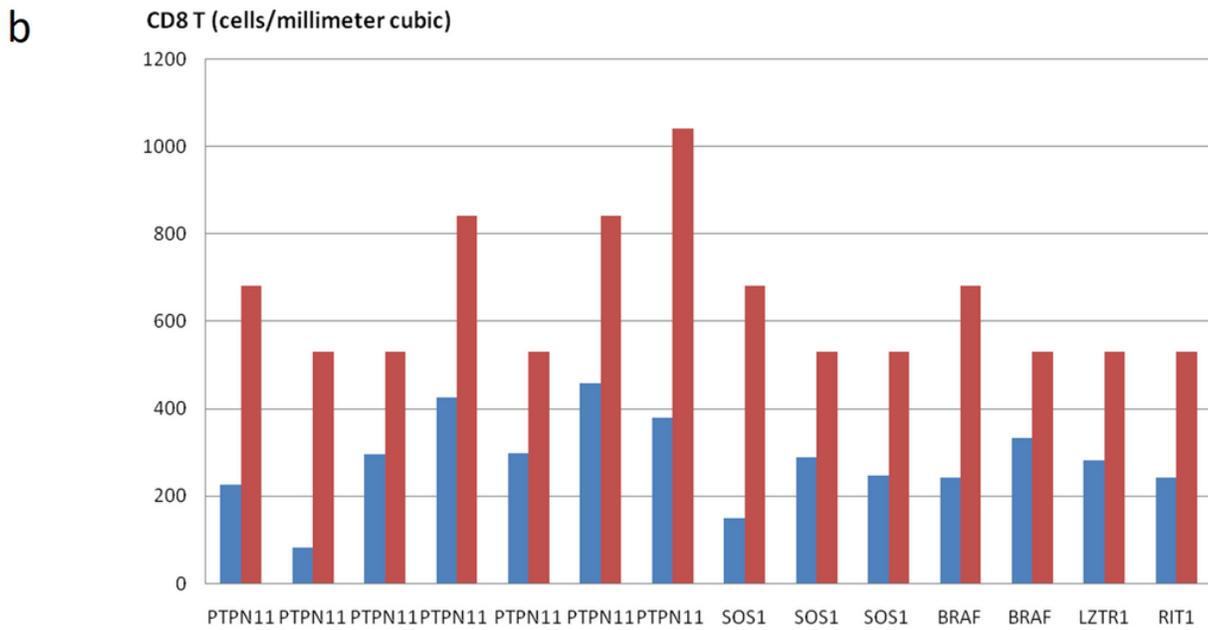
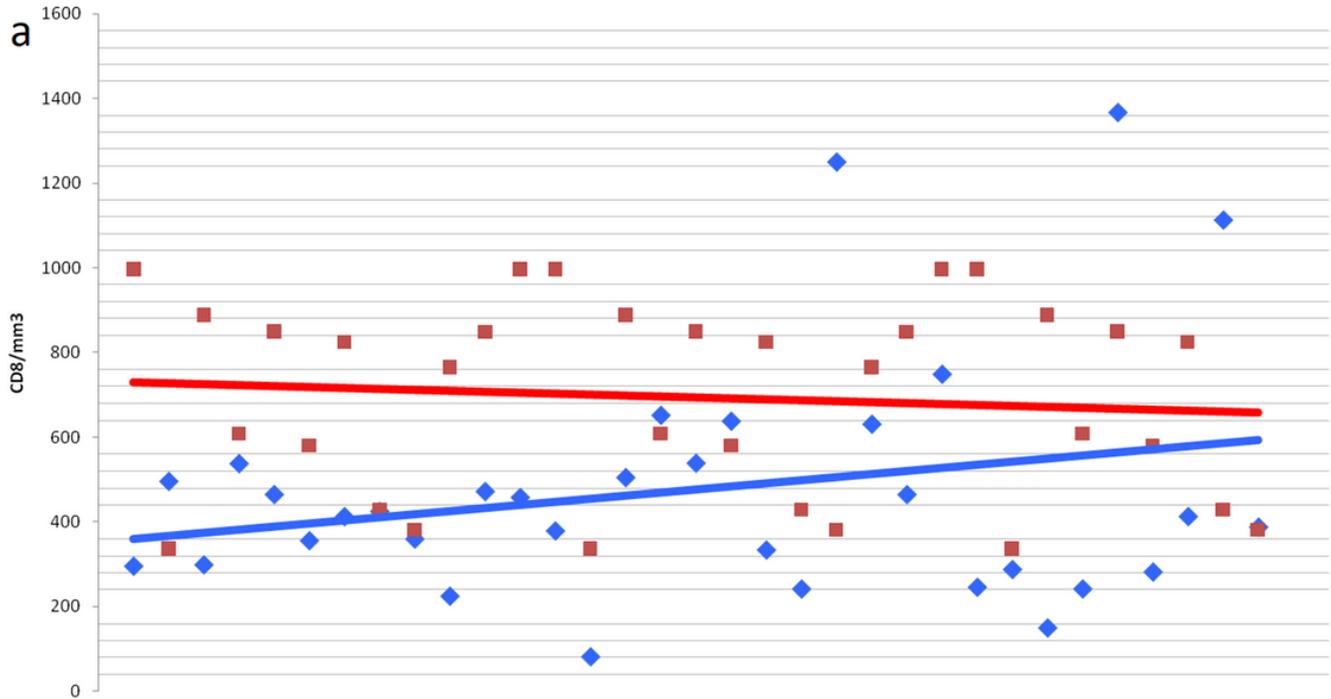


Figure 1

a. CD8 T cells values in patients (blue square) and controls (red diamond) b. CD8 T cells values in patients compared to normal value for age

Supplementary Files

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