BMJ Open

The possible link between coeliac and Kawasaki diseases in Brazil: a cross-sectional study

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To cite: dos Santos Domingues A, Selleski N, Uenishi RH, *et al*. The possible link between coeliac and Kawasaki diseases in Brazil: a cross-sectional study. *BMJ Open* 2018;**8**:e018803. doi:10.1136/bmjopen-2017-018803

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2017-018803).

Received 21 August 2017
Revised 27 November 2017
Accepted 7 December 2017

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ABSTRACT

Background Kawasaki disease (KD) is a self-limited acute systemic vasculitis of unknown aetiology that predominantly affects infants and young children eventually associated with immunological abnormalities. Coeliac disease (CD) is an inflammatory autoimmune disease characterised by a permanent gluten intolerance, which affects genetically susceptible individuals of any age group, and can cause intestinal and systemic symptoms. Association of CD with KD has been previously described in a single study that disclosed a surprisingly high prevalence of CD in children with a history of KD.

Objective To confirm the existence of a higher prevalence of CD among individuals with a history of KD, which would turn the screening for CD in patients with history of KD highly advisable.

Setting Children with history of KD, diagnosed and followed at the Rheumatology Clinic of the Children's Hospital of Brasilia (Brasilia, Brazil).

Participants This study included 110 children with history of KD and a control group composed of 110 presumably healthy children.

Interventions Participants underwent anti-transglutaminase and anti-endomysial antibodies tests and genetic typing for the presence of CD predisposing alleles (HLA-DQ2 and DQ8). Jejunal biopsy was performed when necessary, according the European Society of Paediatric Gastroenterology, Hepatology and Nutrition guidelines.

Results Diagnosis of CD was confirmed in one (0.91%) patient with KD by positive serological tests, presence of predisposing alleles and CD typical lesions on duodenal biopsy. All serological tests were negative among the controls. The prevalence of CD predisposing alleles among patients with KD was 29.09%, similar to the prevalence found among controls, 33.64%.

Conclusion The detected CD prevalence (0.91%) does not confirm the existence of an association between KD and CD since this prevalence is similar to that found in the general population (≃1%).

Strengths and limitations of this study

- ► The results of the current study are based on serological, genetic and biopsy results (when required).
- ► Statistical analysis of HLA typing suggests that patients with Kawasaki disease (KD) do not have a higher prevalence of coeliac disease.
- ► The relatively small number of subjects in our study can be considered a limitation; however, KD is not a frequent disorder in Brazil and most likely underdiagnosed.

INTRODUCTION

Kawasaki disease (KD) is an acute, primarily paediatric (80% in children between 6 months and 4 years), self-limited systemic vasculitis that results in coronary artery abnormalities in up to 25% of untreated children.[1] Although the initial description of its aetiology was made almost 50 years ago, the trigger is still unknown, and its diagnosis depends essentially on clinical data since there are no reliable tests available.[2]

Epidemiological data from surveys conducted mainly in Japan show that incidence of KD depends on a variety of factors. Cases of the disorder tend to cluster geographically and by season, and it has been suggested that infectious agent(s) may be involved, leading to an abnormal immunological response in genetically susceptible individuals. The existence of an underlying genetic factor is suggested by the higher incidence in the Asian population and by increased risk of the disease in patient's close relatives.[3] A comparison with the general population showed that the risk of KD is 10 –30 times higher in siblings of patients with KD[2] and the odds of having sibling cases are significantly increased in patients with parental history of KD.[4] Analysis of KD seasonality of extratropical regions in the Northern hemisphere discloses a defined seasonal structure with higher number of cases in the winter and low number in the summer.[5] These seasonal variations were also tentatively associated with tropospheric wind patterns originating in central Asia and traversing the North Pacific suggesting that an environmental trigger for KD could be windborne.[6]

Coeliac disease (CD) is a systemic inflammatory autoimmune disease characterised by a permanent intolerance to gluten in genetically susceptible individuals. CD has a complex pathogenesis characterised by a strong interplay of environmental, genetic and immunological factors.[7] Serological screening is the main diagnostic test for CD, mainly based on the presence of IgA anti-transglutaminase antibodies (IgA-tTG) that has a sensitivity of 98%, and IgA anti-endomysium antibodies (IgA-EMA), which has a specificity of 99%–100% in subjects positive for IgA-tTG.[8] CD has a strong genetic association with the haplotype HLA-DQ2 found in 90%–95% of patients with CD, and haplotype HLA-DQ8 in the remaining subjects.[7 9]

CD frequently occurs in association with other autoimmune diseases such as type 1 diabetes mellitus (T1DM), Hashimoto's thyroiditis, rheumatoid arthritis and Sjögren syndrome among others.[7 10] An association between CD and KD was previously described in a single research paper that disclose a surprisingly high prevalence of CD among children with history of KD.[11] This study evaluated 90 Italian children (57 boys and 33 girls, median age 5.2 years) and 150 controls, matched for age and sex. The analysis of the results disclosed some inconsistencies regarding an undoubtable diagnosis of CD among the children with a history of KD, mainly due to the low levels of CD specific antibody markers and the lack of details of the intestinal–mucosal biopsy findings.

Consequently, the main objective of the present study was to attempt to replicate this surprisingly high prevalence of CD in a group of Brazilian children with a history of KD. Additionally, to conclusively clarify the possible existence of an increased predisposition to CD in children with a history of KD, we also performed a genetic screening to determine the frequency of HLA predisposing alleles for CD among the KD study group.

PATIENTS AND METHODS

The study included a convenience sample of 110 children with a history of KD, diagnosed and followed at the Rheumatology Clinic of the Children's Hospital of Brasilia (Brasilia, Brazil) from June 2014 to June 2016. KD diagnosis was established according to the American Academy of Pediatrics and American Heart Association's criteria.[12] The diagnosis of CD was achieved according to the revised guidelines of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN).[13] The study complied with the principles of the latest Declaration of Helsinki.[14] After extensive explanation of the purpose and procedures involved in the research, a written informed consent was obtained from the children's parents or caregivers. The study group consisted of 110 children with a history of KD (66 males, 44 females, mean age: 3 years 8 months, median: 3 years, age range: 2–9 years). At the time of the screening for CD, the mean age of the group was 6 years, median: 5 years, age range: 4–11 years. The control group was composed of 110 children without a history of KD (47 males, 63 females, mean age: 7 years 4 months, median: 7 years 3 months, age range: 2–15 years) attended at the Central Clinical Laboratory of the Brasilia University Hospital for blood investigations performed for routine health checkups, suspected or recurrent infections or preoperative tests.

Serum samples from both groups were tested to determine IgA-anti-tTG levels by enzyme-linked immunosorbent assay (QUANTA Lite h-tTG IgA ELISA, INOVA Diagnostics, San Diego, California, USA) and IgA-EMA by indirect immunofluorescence assay (NOVA Lite Monkey Esophagus IFA-INOVA Diagnostics, San Diego, California, USA) following manufacturer's instructions. Duodenal biopsies were performed in the patients who were tested positive for both serological examinations.

Genomic DNA was extracted from each whole blood sample using Illustra Blood Genomic Prep Mini Spin kit (GE Healthcare, Buckinghamshire, UK) according to manufacturer's instructions. The presence or absence of CD *HLA-DQ* predisposing alleles (*DQA1*05-DQB1*02* and *DQA1*03-DQB1*03:02*, which code for class II MHC DQ2 and DQ8 molecules, respectively) was tested in all samples using real-time PCR (qPCR; StepOnePlus Syste-Applied systems). PCR reaction was performed according to Selleski *et al*[15] for the genotyping of DQA1*05, DQB1*02 and DQA1*03. Typing of the *DQB1*03:02* allele was performed as described by Profaizer *et al*.[16]

Statistical analysis was performed using GraphPad Prism V.7 for Windows. Fisher's exact test was chosen to compare data between groups, only results with $P<0.05$ were considered significant. Statistical analysis included the 't-test' for continuous variables and 'Fischer's exact test' for prevalence.

RESULTS

Diagnosis of CD was obtained in one 5-year-old boy (1/110%–0.91%) that disclosed elevated titres of IgA-tTG (114 U; normal value ≤20 U/mL) and strong positive IgA-EMA test (positive to a dilution of 1:160). Both HLA CD predisposing alleles for CD (HLA DQ2 and DQ8) were detected on genetic analysis. Finally, CD diagnosis was confirmed by biopsy of mucosal samples obtained from the duodenal bulb and descending duodenum that showed a Marsh-3A degree of villous atrophy. His clinical evaluation did not show any signs or classic symptoms that could have suggested CD. Our routinely performed investigations on first-degree relatives of patients with CD disclosed that his older sister (age 13) had positive serological tests (IgA-tTG: 94 U/mL and IgA-EMA IgA positive to a dilution of 1:80) and duodenal biopsy compatible with Marsh-I degree of villous atrophy. All serological tests (IgA-EMA and IgA-tTG) performed on the control group yielded negative results.

The genetic screening of the children with KD disclosed a total of 32 (29.09%) carriers of CD predisposing variants. Three children (2.73%) were positive for both HLA-DQ2 and DQ8, 15 (13.64%) disclosed only HLA-DQ2 (DQA1*05, DQB1*02) and the HLA-DQ8

Table 1 Results of the real-time PCR analysis (qPCR) for DQ2 and DQ8 coeliac disease predisposing alleles among the children with Kawasaki disease and controls

	Kawasaki group (n=110)		Controls (n=110)		
Genotype	N	%	N	%	P value
DQ2/DQ8	3	2.73	1	0.91	0.621
DQ2	15	13.64	20	18.18	0.463
DQ8	14	12.73	16	14.5	0.844
Total	32	29.09	37	33.64	0.561

variant (DQA1*03, DQB1*03: 02) was found in 14 (12.73%). HLA typing of the control group revealed a total of 37 (33.64%) predisposing variants. A comparison of the results obtained from the children with KD and controls can be seen in table 1.

DISCUSSION

Abnormal immunological response elicited by gluten-derived proteins can lead to the production of several different autoantibodies, which affect distinct systems and justify the frequent association of CD with other autoimmune diseases. Additionally, CD often display a pronounced genetic overlapping with other autoimmune diseases.[7] Consequently, patients with CD frequently exhibit concurrent autoimmune diseases, conversely patients with other autoimmune diseases may also be affected by CD, particularly those with T1DM or thyroid disease such as Hashimoto thyroiditis and Graves' disease.[7] T1DM is probably the most frequent disorder associated with CD, since 5%–8% of patient with T1DM have CD and vice versa.[7 17 18] This close association has been ascribed to the same HLA pattern, namely HLA-DQ2 and/or DQ8, which predisposes individuals to both disorders.[19] Currently, the positions of the ESPGHAN is that paediatric endocrinologists should screen all patients with T1DM for CD soon after diabetes is diagnosed, since manifestations of CD are often subtle, making early screening extremely relevant.[13 18]

On the other hand, the association of KD with other autoimmune diseases has rarely been observed and has been restricted to case reports; however, a single screening study conducted in Italy stands out as an exception. Their results yielded a surprisingly high prevalence (5.5%) of CD among children with a history of KD.[20] This is a very high prevalence, comparable with the prevalence of 5.6% found among Sahrawi children living in the Western part of the Sahara desert, which is presently still considered the highest worldwide prevalence of CD.[21] Such a high prevalence would place the risk of CD in patients with a history of KD at the same level of any other group considered highly prone to CD, as is the case for T1DM, Down's syndrome and first-degree relatives of patients with CD. Consequently, this would turn screening for CD in all children with history of KD mandatory.

The relatively small number of subjects in our study can be considered a limitation; however, KD is not a frequent disorder in Brazil and most likely underdiagnosed, making the recruitment a challenge. Also, a meaningful sample calculation would not be possible because the prevalence of KD in Brazil is unknown.

In our study, only one patient (0.91%) among the 110 children with a history of KD had a confirmed diagnosis of CD. No case of CD was found among controls. The prevalence found in the KD group is similar to that found worldwide in the general population which is approximately 1%.[22–24]

Genetic typing for the presence of predisposing variants among KD children and controls yielded similar results with 29.09% of predisposing variants among children with KD and 33.64% among controls. Genetic factors are necessary but not sufficient to development CD since as many as 20%–40% healthy subjects in the general population also carry either HLA-DQ2 or HLA-DQ8 haplotype or a combination of both, but only 3%–4% of that population will eventually develop CD.[10 25] Therefore, the value of HLA typing in CD is mainly considered for its negative predictive value since this disorder in the absence of these genetic markers is extremely rare.[7 10 26] Consequently, the comparison of the percentage of predisposing alleles between KD group and control leads inevitably to the conclusion that both groups have similar predisposition to CD, which is also similar to that found in the general population.

CONCLUSION

Although our study has the weakness of being based on a small number of patients and controls, our result suggests that children with a history of KD neither have an increased prevalence nor an heightened predisposition to CD.

Contributors LG and CBP conceptualised the study and critically revised the protocol. CMRdM contributed with data acquisition and data analysis. AdSD and NS drafted the manuscript. NS worked on the acquisition and interpretation of HLA data. RHU developed the methodological approach and worked on the acquisition and interpretation of serological assays. AdSD, NS and RHU contributed to the interpretation of data and critically revised the manuscript. LG and CBP are the guarantors of the study and were responsible for the final version of the manuscript. All authors read and approved the final version of the manuscript.

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent Parental/guardian consent obtained.

Ethics approval Ethics Committee of the Children's Hospital of Brasilia.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The statistical analysis and examination results of deidentified participants of Kawasakidisease group and control group that were used and analysed during the current study are available from the corresponding author on reasonable request. Please allow 10 business days for data to be emailed.

REFERENCES

1. Yim D, Curtis N, Cheung M, *et al*. Update on Kawasaki disease: epidemiology, aetiology and pathogenesis: an update on Kawasaki disease: part I. *J Paediatr Child Health* 2013;49:704–8.
2. Takahashi K, Oharaseki T, Yokouchi Y. Update on etio and immunopathogenesis of Kawasaki disease. *Curr Opin Rheumatol* 2014;26:31–6.
3. Onouchi Y, Tamari M, Takahashi A, *et al*. A genomewide linkage analysis of Kawasaki disease: evidence for linkage to chromosome 12. *J Hum Genet* 2007;52:179–90.
4. Uehara R, Yashiro M, Nakamura Y, *et al*. Kawasaki disease in parents and children. *Acta Paediatr* 2003;92:694–7.
5. Burns JC, Herzog L, Fabri O, *et al*. Seasonality of Kawasaki disease: a global perspective. *PLoS One* 2013;8:e74529.
6. Rodó X, Ballester J, Cayan D, *et al*. Association of Kawasaki disease with tropospheric wind patterns. *Sci Rep* 2011;1.
7. Lundin KE, Wijmenga C. Coeliac disease and autoimmune disease-genetic overlap and screening. *Nat Rev Gastroenterol Hepatol* 2015;12:507–15.
8. Tran TH. Advances in pediatric celiac disease. *Curr Opin Pediatr* 2014;26:585–9.
9. Louka AS, Sollid LM. HLA in coeliac disease: unravelling the complex genetics of a complex disorder. *Tissue Antigens* 2003;61:105–17.
10. Sollid LM, Lie BA. Celiac disease genetics: current concepts and practical applications. *Clin Gastroenterol Hepatol* 2005;3:843–51.
11. Stagi S, Simonini G, Ricci L, *et al*. Coeliac disease in patients with Kawasaki disease. Is there a link? *Rheumatology* 2006;45:847–50.
12. Newburger JW, Takahashi M, Gerber MA, *et al*. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Pediatrics* 2004;114:1708–33.
13. Husby S, Koletzko S, Korponay-Szabó IR, *et al*. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012;54:136–60.
14. World Medical Association.. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Bull World Health Organ* 2001;79:373–4.
15. Selleski N, Almeida LM, Almeida FC, De AFC, *et al*. Simplifying celiac disease predisposing HLA-DQ alleles determination by the real time PCR method. *Arq Gastroenterol* 2015;52:143–6.
16. Profaizer T, Eckels D, Delgado JC. Celiac disease and HLA typing using real-time PCR with melting curve analysis. *Tissue Antigens* 2011;78:31–7.
17. Elfström P, Sundström J, Ludvigsson JF. Systematic review with meta-analysis: associations between coeliac disease and type 1 diabetes. *Aliment Pharmacol Ther* 2014;40:1123–32.
18. Gabriel L, Pettei MJ, Carey DE, *et al*. Pediatric endocrinologists' practices in screening for celiac disease in patients with type 1 diabetes. *Journal of Diabetes Research and Clinical Metabolism* 2015;4:1.
19. Volta U, Tovoli F, Caio G. Clinical and immunological features of celiac disease in patients with Type 1 diabetes mellitus. *Expert Rev Gastroenterol Hepatol* 2011;5:479–87.
20. Stagi S, Simonini G, Ricci L, *et al*. Coeliac disease in patients with Kawasaki disease. *Is there a link? Rheumatol Oxf Engl* 2006;45:847–50.
21. Catassi C, Rätsch IM, Gandolfi L, *et al*. Why is coeliac disease endemic in the people of the Sahara? *Lancet* 1999;354:647–8.
22. Murch S, Jenkins H, Auth M, *et al*. Joint BSPGHAN and Coeliac UK guidelines for the diagnosis and management of coeliac disease in children. *Arch Dis Child* 2013;98:806–11.
23. Crovella S, Brandao L, Guimaraes R, *et al*. Speeding up coeliac disease diagnosis in the developing countries. *Dig Liver Dis* 2007;39:900–2.
24. Catassi C, Anderson RP, Hill ID, *et al*. World perspective on celiac disease. *J Pediatr Gastroenterol Nutr* 2012;55:494–9.
25. Verdu EF, Caminero A. How infection can incite sensitivity to food. *Science* 2017;356:29–30.
26. Rashid M, Lee J. Serologic testing in celiac disease. *Can Fam Physician* 2016;62:38–43.