

**Title**

HLA DRB1\*0415: a new possible genetic susceptibility factor for Hirata's Disease.

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**Disclosure summary**

The authors declare that they have no conflict of interest.

**Author contributions**

All authors contributed in the realization of this article, performing literature search, writing or revising the manuscript.

**Abstract**

**Context** Hirata's Disease (HD) is a rare autoimmune cause of hypoglycemia. Patients suffering from this condition have a genetic predisposition, determined by HLA DR4, with some differences in the worldwide population. In Caucasians HLA DRB1\*0403 is the most frequent susceptibility background on which some drugs play as triggers.

**Case Description** We reported the case of a woman with several hypoglycemic episodes, characterized by high insulin and c-peptide levels. Biochemical and morphological exams excluded a neuroendocrine tumor. Hirata's Disease was diagnosed according to insulin autoantibodies (IAA) positivity and patient's history, particularly about drugs taken. The HLA analysis revealed DRB1\*0415 allele.

**Conclusions** We found a potential new predisposing factor for HD, HLA DRB1\*0415 allele, never described before as genetic background to Insulin Autoimmune Syndrome in Caucasians.

**Keywords:** hypoglycemia, alpha lipoic acid, insulin autoantibodies, Insulin Autoimmune Syndrome.

## **Introduction**

Insulin Autoimmune Syndrome (IAS), also known as Hirata's Disease (HD), is a rare cause of hypoglycemia, most frequent in Japan where it represents the third cause of hypoglycemia [1], with up to 380 reported cases [2].

This autoimmune syndrome is characterized by spontaneous hypoglycemic episodes with insulin autoantibodies (IAA) positivity in patients who have never received exogenous insulin treatment. The specific mechanism by which IAA lead to hypoglycemic episodes is not clear. The main hypothesis is that insulin produced by pancreatic  $\beta$ -cells after food intake is bound by IAA, creating IAA-insulin complexes which are a circulating insulin reserve. During the fasting period, when circulating insulin levels are reduced, the insulin release from the IAA could be responsible of the hypoglycemic episodes [3]. Another hypothesis is that IAA could induce the production of anti-idiotypic antibodies which might bind insulin receptors and be functionally similar to circulating insulin.

Like other autoimmune disorders, IAS is characterized by a genetic predisposition associated with the Human Leukocyte Antigen (HLA) DR4. In Japanese population, particularly, there is a strong association between IAS and HLA DRB1\*0406, and less likely with DRB1\*0403 and DRB1\*0407. The higher prevalence in Japan could be explained by the different distribution of HLA DR4 alleles around the world. Indeed, HLA-DRB1\*0406 is prevalent in Eastern Asian population while HLA DRB1\*0403 is mainly expressed in Caucasians [4].

On this genetic background the use of some drugs can play an important role. Various medications are reported to lead to IAS, and all of them are sulfhydryl compounds able to reduce the disulfide bridges between the two insulin chains, increasing the insulin immunogenicity [2].

## **Case report**

A 76-year-old woman with type 2 diabetes (T2DM) was admitted to the Inpatient Endocrinology Clinic on February 2018 because of recurrent hypoglycemic episodes. She had never used insulin to treat her T2DM. Metformin was prescribed at the diagnosis, but she spontaneously suspended it when the first hypoglycemic episode occurred.

During the hospitalization, several symptomatic and spontaneous hypoglycemic episodes occurred, all characterized by high levels of insulin (47.1-50.4-46.5-53.7-43.3  $\mu$ UI/ml – normal values <30.0) and c-peptide (17.8-17-16-19.1-14.7 ng/ml – reference range 1.1-3.5). Cortisol, ACTH and GH values were in the normal range. The factitious use of sulfonylureas was excluded.

In order to exclude an insulin-secreting tumor, Neuron Specific Enolase (NSE) was measured but was in the normal range (14.4 ng/ml). Chromogranin A was elevated (1511 ng/ml) during proton pump inhibitor treatment but decreased to 101.4 ng/ml after removing the pharmacological interference. As morphological study of the pancreas a contrast CT scan, an echoendoscopy and an abdomen MRI scan were performed, but no lesion suspected of neuroendocrine tumor was identified. A 68-Ga-DOTATOC PET excluded the presence of somatostatin receptor-positive lesions.

For the persistence of symptomatic hypoglycemic episodes during the hospitalization, diazoxide was prescribed and continued after discharge from hospital, obtaining a good control of symptoms and blood glucose values.

In the next few months, blood glucose levels progressively increased, so the diazoxide dose was reduced accordingly. In June 2018 the patient was admitted again to the Inpatient Endocrinology Clinic where diazoxide was suspended. At the end of a prolonged fasting test asymptomatic low blood glucose values (53-56-50 mg/dl) were observed but associated with normal insulin (15.1-14.1-16.1  $\mu$ UI/ml) and c-peptide (0.9-0.9-0.9 ng/ml) levels, resulting in a normal response.

Suspecting IAS, IAA were measured and resulted elevated (45.5 AU/ml; normal values <0.4). The hypothesis of IAS was sustained by the medication history because she had taken a dietary supplement containing alpha lipoic acid (ALA) to treat her diabetic neuropathy for a few weeks before the first hospitalization.

Glycated haemoglobin (HbA1c) was 82 mmol/mol, suggesting poorly-controlled diabetes and spontaneous remission of hypoglycemia.

After a few months, the patient was asymptomatic without diazoxide. HbA1c was 51 mmol/mol in the absence of pharmacological interference, and IAA gradually decreased (35.8-16.6 AU/ml) (Table 1). Further on, blood glucose profile showed values in upper normal limits without any treatment.

The HLA analysis revealed HLA DRB1\*0415 allele, never described before in association with IAS, to our knowledge.

## Discussion

IAS is a rare autoimmune condition, especially in Europe, but potentially under- or misdiagnosed. The differential diagnosis of symptomatic hypoglycemia may be hard, including various causes such as sepsis, hepatic, renal, or cardiac failure, drugs, and less frequently insulinoma, and insulin like growth factor-II secreting tumors [5]. In this context the IAS diagnosis could be more difficult because of clinical features simulating insulinoma. There are some case reports of IAS being misdiagnosed as cases of insulinoma and therefore undergoing pancreas surgery, not only in the past, but even more recently [6]. Based on its role in the differential diagnosis, the IAA measurement is now recommended by the clinical practice guideline [5,7].

In our case the diagnostic hypothesis was raised by high insulin levels associated with normal pancreas morphology, progressive elevation of blood glucose levels during diazoxide, and finally spontaneous remission. The hypothesis was confirmed assessing IAA, which turned positive. After diagnosis, it would be important to identify the responsible for the IAA development in order to avoid further exposure. In fact, because of their trigger role, drugs must be carefully investigated. Drug history, including dietary supplement, could be crucial for suspecting IAS. A lot of sulfhydryl compounds are commonly used to treat various illnesses [2].

The first case of IAS associated with ALA assumption was reported in Japan in 2003 [2]. Between 2004 and 2007, 17 cases of IAS after ALA were described in Japan; 12 of them were analyzed for HLA, reporting DRB1\*0406 and DRB1\*0403 in 10 and 2 patients, respectively [2]. The first Italian IAS after ALA was described in 2011, with HLA DRB1\*0406 [8]. ALA is widely used as nutritional supplement for its antioxidant properties and as adjuvant treatment for diabetic neuropathy. ALA assumption could be very hard to investigate because many patients like ours do not consider it as a pharmacological product but a natural dietary supplement, so it won't be mentioned in the medication history.

The different prevalence of IAS around the world, despite the widespread use of the same sulfhydryl compounds, could have a genetic base, secondary to the distribution of HLA DR4 alleles in Japanese vs. Caucasian population [4]. The Italian cases reported until January 2019 mostly expressed HLA DRB1\*0403 (9/15), and less frequently HLA DRB1\*0406 (2/15), and HLA DRB1\*0407 (1/15). In the remaining 3 patients HLA assessment was not performed [9,10,11]. Going into details, out of the 15 Italian patients with IAS, 10 were described after taking ALA; HLA DRB1\*0403 and DRB1\*0406 were identified in 8 and 2 of them, respectively. To our knowledge, this is the first case characterized by HLA DRB1\*0415, suggesting that other HLA DR4 alleles could lead to IAS. (Table 2).

## Conclusion

In conclusion, IAS should be included in the differential diagnosis of hypoglycemia according to the clinical practice guideline, in order to avoid under- or misdiagnosis.

Although HLA analysis cannot be reasonably performed in every patient taking sulfhydryl compounds, it would be interesting to evaluate the genetic predisposition in advance, at least for research purposes.

The increasing number of ALA-induced hypoglycemic episodes calls for caution in prescribing these compounds and assessing self-medication practices when hypoglycemic episodes are not explained otherwise.

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Time	Plasma glucose (mg/dl)	HbA1c (mmol/mol)	Insulin ( $\mu$ UI/ml)	C-peptide (ng/ml)	IAA (AU/ml)	Cromogranin A (ng/ml)	NSE (ng/ml)
First Admission	21	44	47,1	17,8	NP	1511	14,4
During Diazoxide	225	82	NP	NP	NP	NP	NP
Second Admission	133	80	NP	NP	45,5	101,4	NP
Fasting test <sup>a</sup>	50	NP	16,1	0,9	NP	NP	NP
After hospital discharge (1 month)	124	75	34,9	2,7	35,8	NP	NP
After hospital discharge (5 months)	127	51	35,6	2,4	16,5	184	NP

**Table 1.** Biochemistry laboratory values of the reported case (NP= Not Performed)

<sup>a</sup> Reported are the lowest plasma glucose during the fasting test and the corresponding levels of Insulin and C-peptide

Patient	Age	Sex	Trigger	Plasma glucose (mg/dl)	Insulin ( $\mu$ UI/ml)	C-peptide (ng/ml)	IAA	HLA	Therapy
1	Pediatric	M	ND	34	ND	ND	ND	ND	NS
2	58	F	ND	20	426	ND	Positive	ND	Plasmapheresis/Prednisone
3	70	F	ALA	24	57	11,9	Positive	HLA-DRB1*0406	Prednisone/Diazoxide
4	40	F	ND	47	43	4,6	Positive	ND	Plasmapheresis
5	75	M	ALA	29	4930	4,1	Positive	HLA-DRB1*0406	Prednisone
6	77	F	ALA	36	8000	7,1	Positive	HLA-DRB1*0403	Prednisone
7	53	M	ALA	26	52900	6,8	Positive	HLA-DRB1*0403	Prednisone
8	40	F	ALA	35	533	5,1	Positive	HLA-DRB1*0403	Fractionated meals
9	70	F	ALA	45	3200	4,8	Positive	HLA-DRB1*0403	Prednisone
10	56	M	ALA	40	2200	6,5	Positive	HLA-DRB1*0403	Prednisone
11	78	F	ND	44	1000	3,1	Positive	HLA-DRB1*0407	Prednisone
12	54	M	ND	52	600	2,9	Positive	HLA-DRB1*0403	Fractionated meals
13	56	F	ALA	21	8500	18,4	Positive	HLA-DRB1*0403	Prednisone
14	66	F	ALA	24	197	3,4	Positive	HLA-DRB1*0403	Prednisone
15	82	F	ALA	34	>1500	4,2	Positive	HLA-DRB1*0403	Prednisone
Present case	76	F	ALA	21	47,1	17,8	Positive	HLA-DRB1*0415	Diazoxide

**Table 2.** Features of the Italian cases of IAS reported so far. (ND= Not Determined, NS= Not Specified, ALA= Alpha Lipoic Acid).