

# SCIENTIFIC DOSSIER

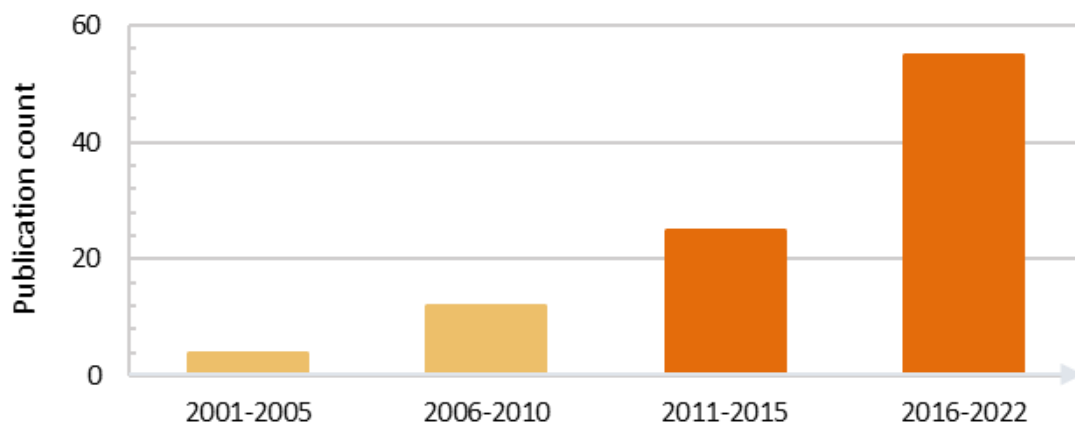
## ON THE DIETARY TREATMENT OF DAO DEFICIENCY SYMPTOMS

‘absence of evidence  
is not evidence of absence’

CARL SAGAN (1934-1996)  
Astrophysicist, science communicator and freethinker

80%  
[2011-2022]

Key words: "DAO Deficiency" and "histamine intolerance"  
Searcher: PubMed  
Database: MEDLINE



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## **1. Diamine Oxidase and Migraine**

**2020 – Associations between migraine, celiac disease, non-celiac gluten sensitivity and activity of diamine oxidase.** K. Griauzdaitė, K. Maselis\*, A. Žvirblienė, A. Vaitkus, D. Jančiauskas, I. Banaitytė-Baleišienė, L. Kupčinskas, D. Rastenytė. Medical Hypotheses 142. **2020**. 109738

**2019 – Association of Diamine oxidase (DAO) variants with the risk for migraine from North Indian population.** Sukhvinder Kaura, Arif Alib, Yaser Siahbalaic, Uzair Ahmadc, Fazila Nargisc, A.K. Pandeyd, Balkirat. **2019** October 2214-5400- / © 2019 Elsevier B.V.

**2019 - Migraines appear more likely to be caused by histamine rather than ethanol.** Schnedl, W. J., & Queissner, R. 2019. European journal of neurology.

**2018 – Diamine oxidase (DAO) supplement reduces headache in episodic migraine patients with DAO deficiency: A randomized double-blind trial.** Joan Izquierdo-Casas; Oriol Comas-Basté; M. Luz Latorre-Moratalla; Marian Lorente-Gascón; Adriana Duelo; M. Carmen Vidal-Carou; Luis Soler-Singla **2018** Clinical Nutrition.

**2018 – Low-histamine diet supplemented with exogenous diamine oxidase enzyme is useful for treating migraine in patients with DAO Deficiency** A.Duelo; M.Berbel; H.Mantecon-Laviguerie; O.Comas-Basté; M.L.Latorre-Moratalla; M.T.Veciana-Noguès; M.C.Vidal-Carou. **2018** Spanish Nutrition Society SEÑ and the Catalan Association of Food Science (ACCA), Spain

**2017 – Low serum diamine oxidase (DAO) activity levels in patients with migraine.** Izquierdo-Casas J1,2, Comas-Basté O3, Latorre-Moratalla ML3, Lorente-Gascón M2, Duelo A4, Vidal-Carou MC5, Soler-Singla L1,2. **2017**- J Physiol Biochem. doi: 10.1007/s13105-017-0571-3.

**2016 - Migraine: A disorder of metabolism?** Kokavec A. (2016). Medical hypotheses.

**2016 – Association of diamine oxidase and histamine N-methyltransferase polymorphisms with presence of migraine in a group of Mexican mothers of children with allergies.** R. Meza-Velázquez, d, F. López-Márquez, b, S. Espinosa-Padilla, c, M. Rivera-Guillén, d, J. Ávila-Hernández, b y M. Rosales-González, \* **2016** Sociedad Española de Neurología. Elsevier España, S.L.U.

**2015 – Diamine Oxidase rs10156191 and rs2052129 Variants Are Associated With the Risk for Migraine.** Elena García-Martín, MD, PhD; Carmen Martínez, MD, PhD; Mercedes Serrador, MD, PhD; Hortensia Alonso-Navarro, MD, PhD; Pedro Ayuso, MD; Francisco Navacerrada, MD; José A. G. Agúndez MD, PhD; Félix Javier Jiménez-Jiménez, MD, PhD. **2015** Headache, January, American Headache Society, ISSN 0017-8748 - doi: 10.1111/head.12493.22

**2014 - Histamine in migraine and brain.** Alstadhaug K. B. (2014). Headache.

**2013 – A randomized doubled blinded trial of treatment with DiAmineOxidase (DAO) in patients with migraine and deficit of enzyme's activity.** J. Izquierdo. D. Mon, M. Lorente, L. Soler Singla. Hospital General de Catalunya, Universitat Internacional de Catalunya, Sant Cugat del Vallès, Spain. **2013** XXI World Congress of Neurology. Wien/Austria. Journal of the Neurological Sciences 333,

**2012 – Diamine Oxidase as a triggering factor for migraine.** J. Izquierdo, LI. Soler, E. Balaguer, D. Mon, Neurology Service. Capió Hospital General de Catalunya. **2012** - 16th Annual Meeting of the Catalan Society of Neurology. 26th Neurology Update Course. 40th Yearly gathering of the Catalan Society of Neurology.

## **2. Diamine Oxidase in Gastroenterology, Respiratory and Pediatrics**

**2023 - Diamine Oxidase Activity Deficit and Idiopathic Rhinitis: A New Subgroup of Non-Allergic Rhinitis?** Mayo-Yáñez M, Díaz-Díaz A, Calvo-Henríquez C, Lechien JR, Vaira LA, Figueroa A. **2023**. *Life* (Basel).

**2022 - The dietary treatment of histamine intolerance reduces the abundance of some histamine-secreting bacteria of the gut microbiota in histamine intolerant women. A pilot study.** Sánchez-Pérez S, Comas-Basté O, Duelo A, Veciana-Nogués MT, Berlanga M, Vidal-Carou MC and Latorre-Moratalla ML. **2022**. *Front. Nutr.*

**2022 - Histamine: A Mediator of Intestinal Disorders—A Review.** Smolinska S, Winiarska E, Globinska A, Jutel M. *Metabolites*. **2022**.

**2022 - Intestinal Dysbiosis in Patients with Histamine Intolerance.** Sánchez-Pérez, S., Comas-Basté, O., Duelo, A., Veciana-Nogués, M. T., Berlanga, M., Latorre-Moratalla, M. L., & Vidal-Carou, M. C. *Nutrients*. **2022**.

**2021 – Relationship between allergic rhinitis and diamine oxidase activity: A preliminary report.** Miguel Mayo-Yáñez, Andrea Díaz-Díaz, Juan C. Vázquez-Barro, Jesús Herranz González-Botas, Angélica Figuerioa, and Carlos S. Martín-Martín. *Allergologie*. **2021**. 187-194

**2021 – Histamine Intolerance Originates in the Gut.** Wolfgang J. Schnedl and Dietmar Enko. *Nutrients*. **2021**. 13, 1262

**2020 – Considering histamine in functional gastrointestinal disorders.** Wolfgang J. Schnedl & Dietmar Enko. *Critical Reviews in Food Science and Nutrition*. **2020**.

**2019 – Diagnostic and Clinical Significance of Serum Levels of D-Lactate and Diamine Oxidase in Patients with Crohn’s Disease.** Jierui Cai, Hong Chen, Meiling Weng, Shuyu Jiang, and Jie Gao. *Gastroenterology Research and Practice*. **2019**. 7 pages

**2018 – Microbial patterns in patients with histamine intolerance.** M. Schink, P.C. Konturek, E. Tietz, W Dieterich, T.C. Pinzer, S. Wirtz, M.F. Neurath, Y. Zopf. *Journal of physiology and pharmacology*. **2018**. 69, 4, 579-593

**2017 – Non-celiac gluten sensitivity: people without celiac disease avoiding gluten – is it due to histamine intolerance?** Wolfgang J. Schnedl, Sonja Lackner, Dietmar Enko, Michael Schenk, Harald Mangge, Sandra J. Holasek. *Inflammation Research*. **2017**

**2017 – Serum Diamine Oxidase in Pseudoallergy in the Pediatric Population** Joanna Kacik, Barbara Wroblewska, Sławomir Lewicki, Robert Zdanowski, and Bolesław Kalicki **2017** *Advances in Medicine, Biology - Neuroscience and Respiration*

**2016 – Association between two polymorphisms of histamine-metabolising enzymes and the severity of allergic rhinitis in a group of Mexican children** R. Meza-Velázquez, F. López-Márquez, S. Espinosa-Padilla, M. Rivera-Guillen, N. Gutiérrez-Díaz, L. Pérez-Armendáriz, M. Rosales-González, **2016** *Allergol Immunopathol* Received 20 November 2015; accepted 29 January

**2014 - Chronic rhinitis and its association with headache frequency and disability in persons with migraine: results of the American Migraine Prevalence and Prevention (AMPP) Study.** Martin, V. T., Fanning, K. M., Serrano, D., Buse, D. C., Reed, M. L., Bernstein, J. A., & Lipton, R. B. (2014). *Cephalalgia: an international journal of headache*.

**2014 - Diamine oxidase activity levels in anorexia nervosa.** Takimoto, Y., Yoshiuchi, K., Shimodaira, S., & Akabayashi, A. (2014). The International journal of eating disorders.

**2013 – Dietary treatment and blood diamineoxidase (DAO) values in patients affected vomiting and other gastrointestinal and neurological symptoms.**Tormo R, Cárdenas G, Seguroola H. Gastroenterology and Nutrition Unit Via Augusta. Barcelona. Spain. **2013** Congress of the European Society for Parenteral and Enteral Nutrition (ESPEN) Leipzig / Germany. Clinical Nutrition, sept. 32, S145

**2013 – Histamine intolerance as a cause of chronic digestive complaints in pediatric patients.** Anotnio Rosell-Camps, Sara Zibetti, Gerardo Pérez-Esteban, Magdalena Vila-Vidal, Laia Ferrés-Ramis and Elisa García-Teresa-García. Hospital Universitario Son Espases. Palma de Mallorca, Balearic Islands. Spain. **2013**. REV ESP ENFERM DIG (Madrid) Vol. 105. N° 4, pp 201-207

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**2003 - The diet factor in pediatric and adolescent migraine.** Millichap, J. G., & Yee, M. M. (2003). Pediatric neurology.

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**2019 - Massive release of the histamine-degrading enzyme diamine oxidase during severe anaphylaxis in mastocytosis patients.** Boehm, T., Reiter, B., Ristl, R., Petroczi, K., Sperr, W., Stimpfl, T., Valent, P., & Jilma, B. 2019. Allergy.

**2019 - Impaired resolution of wheals in the skin prick test and low diamine oxidase blood level in allergic patients.** Wagner, A., Buczyłko, K., Zielińska-Bliźniewska, H., & Wagner, W. 2019. Postepy dermatologii i alergologii.

**2018 - A Histamine-Free Diet Is Helpful for Treatment of Adult Patients with Chronic Spontaneous Urticaria.** Son JH, Chung BY, Kim HO, Park CW. Ann Dermatol. 2018

**2017 - A Popular myth - low-histamine diet improves chronic spontaneous urticaria - fact or fiction?.** Wagner, N., Dirk, D., Peveling-Oberhag, A., Reese, I., Rady-Pizarro, U., Mitzel, H., & Staubach, P. (2017). Journal of the European Academy of Dermatology and Venereology.

**2016 – Histamine intolerance in patients with chronic spontaneous urticaria.** F. Siebenhaar, A. Melde, M. Magerl, T. Zuberbier, M.K. Church, M. Maurer. JEADV. **2016**

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**2009 – Exogenous Histamine Aggravates Eczema in a Subgroup of Patients with Atopic Dermatitis.** Margitta WORM, Eva-Maria FIELDER, Sabine DÖLLE, Tania SCHINK, Wolfgang HEMMER, Reinhart JARISCH, and Torsten ZUBERBIER. Acta Derm Venereol. **2009**. 89: 52-56

**2006 – Evidence for a reduced histamine degradation capacity in a subgroup of patients with atopic eczema.** Laura Maintz, MD, Said Benfadal, Jean-Pierre Allam, MD, Tobias Hagemann, MD, Rolf Fimmers PhD, and Natalija Novak, MD. *J Allergy Clin Immunol.* **2006.** Volume 117, Number 5: 1106-1112

#### **4. Diamine Oxidase on Menstrual cycle and Pregnancy**

**2017- Pregnancy-associated diamine oxidase originates from extravillous trophoblasts and is decreased in early-onset preeclampsia.** Velicky, P., Windsperger, K., Petroczi, K. et al. 2018. *Scientific Reports.*

**2012 – Effect of the menstrual cycle on serum diamine oxidase levels in healthy women** Yasuhiro Hamada, Yurika Shinohara, Miyuki Yano, Mari Yamamoto, Misa Yoshio, Kumiko Satake, Akiyo Toda, Midori Hirai, Makoto Usami. **2012** *The Canadian Society of Clinical Chemists.* Published by Elsevier Inc

**2008 – Effects of histamine and diamine oxidase activities on pregnancy: a critical review.** Laura Maintz<sup>1</sup>, Verena Schwarzer<sup>2</sup>, Thomas Bieber<sup>1</sup>, Katrin van der Ven<sup>2</sup> and Natalija Novak<sup>1</sup> <sup>1</sup> Department of Dermatology and Allergology, University of Bonn, Germany. <sup>2</sup> Department of Obstetrics and Gynaecology, University of Bonn, **2008** Germany- May 22, *European Society of Human Reproduction and Embryology.*

#### **5. Diamine Oxidase as a coadjuvant in Cancer**

**2022 – Serum diamine oxidase activity derived from response to chemotherapy affects adverse events and serum amino acid levels.** Yuta Sato, Yoshihiro Tanaka, Takeharu Imai, Naoki Okumura, Nobuhisa Matsushashi, Takao Takahashi, Toshio Shimokawa, Kazuhiro Yoshida. **2022.** *Support Care Cancer.*

**2015 – Serum diamine oxidase activity as a predictor of gastrointestinal toxicity and malnutrition due to anticancer drugs.** Jinsei Miyoshi, Hiroshi Miyamoto, Takahiro Goji, Tatsuya Taniguchi, Tetsu Tomonari, Masahiro Sogabe, Tetsuo Kimura, Shinji Kitamura, Koichi Okamoto, Yasuteru Fujino, Naoki Muguruma, Toshiya Okahisa and Tetsuji Takayama. **2015** *Journal of Gastroenterology and Hepatology, May 2015, Vol. 30 Issue 6*

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**2012 - Plasma diamine oxidase activity is a useful biomarker for evaluating gastrointestinal tract toxicities during chemotherapy with oral fluorouracil anti-cancer drugs in patients with gastric cancer.** Namikawa, T., Fukudome, I., Kitagawa, H., Okabayashi, T., Kobayashi, M., & Hanazaki, K. (2012). *Oncology.*

#### **6. Histamine in ADHD**

**2020 – ADHD in school-age children is related to infant exposure to systemic H1-antihistamines.** Saskia Fuhrmann, Falko Tesch, Marcel Romanos, Susanne Abraham, Jochen Schmitt. **2020.** *Allergy / Volume 75, Issue 11*

**2018 – Attention deficit–hyperactivity disorder is associated with allergic symptoms and low levels of hemoglobin and serotonin.** Liang-Jen Wang, Ya-Hui Yu, Ming-Ling Fu, Wen-Ting Yeh, Jung-Lung Hsu, Yao-Hsu Yang, Wei J. Chen, Bor-Luen Chiang & Wen-Harn Pan. **2018.** Nature, Scientific Reports

**2017 – Increased attention-deficit/hyperactivity symptoms in atopic dermatitis are associated with history of antihistamine use.** J Schmitt, A Buske-Kirschbaum, F Tesch, K Trikojat, V Stephan, S Abraham, A Bauer, K Nemat, F Plessow, V Roessner. **2017.** Allergy, 73(3), 615-626

## **7. Diamine Oxidase Deficiency / Histamine Intolerance**

**2022 - Serum Diamine Oxidase Values, Indicating Histamine Intolerance, Influence Lactose Tolerance Breath Test Results.** Wolfgang J Schnedl, Nathalie Meier-Allard, Simon Michaelis, Sonja Lackner, Dietmar Enko, Harald Mangge, Sandra J Holasek. Nutrients. 2022.

**2022 - Histamine Intolerance-A Kind of Pseudoallergic Reaction.** Zhao, Y., Zhang, X., Jin, H., Chen, L., Ji, J., & Zhang, Z. Biomolecules, 2022.

**2021 – Histamine Intolerance Originates in the Gut.** Wolfgang J. Schnedl and Dietmar Enko. Nutrients. **2021.** 13, 1262

**2021 – Food Intolerance: The Role of Histamine.** Yulia O. Shulpekova, Vladimir M. Nechaev, Irina R. Popova, Tatiana A. Deeva, Arthur T. Kopylov, Kristina A. Malsagova, Anna L. Kaysheva and Vladimir T. Ivashkin. **2021.** Nutrients, 13, 3207

**2021 – Histamine Intolerance – The More We Know the Less We Know. A review.** Martin Hrubisko, Radoslav Danis, Martin Huorka and Martin Wawruch. Nutrients. **2021**

**2021 – Histamine Intolerance in Children: A Narrative Review.** Wojciech Nazar, Katarzyna Plata-Nazar, Katarzyna Sznurkowska and Agnieszka Szlagatys-Sidorkiewicz. Nutrients. **2021.**

**2019 – Evaluation of symptoms and symptom combinations in histamine intolerance.** Wolfgang J. Schnedl, Sonja Lackner, Dietmar Boko, Michael Schenk, Sandra J. Holasek, Harald Mangge. Intest Res. **2019;** 17(3):427-433

**2019 - Histamine-reduced diet and increase of serum diamine oxidase correlating to diet compliance in histamine intolerance.** Lackner, S., Malcher, V., Enko, D., Mangge, H., Holasek, S. J., & Schnedl, W. J. 2019. European journal of clinical nutrition.

**2017 - Tyramine and histamine risk assessment related to consumption of dry fermented sausages by the Spanish population.** Latorre-Moratalla, M. L., Comas-Basté, O., Bover-Cid, S., & Vidal-Carou, M. C. (2017). Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association.

**2017 - Serum diamine oxidase activity is associated with lactose malabsorption phenotypic variation.** Enko, D., Kriegshäuser, G., Halwachs-Baumann, G., Mangge, H., & Schnedl, W. J. (2017). Clinical biochemistry.

**2016 - Concomitant Prevalence of Low Serum Diamine Oxidase Activity and Carbohydrate Malabsorption.** Enko, D., Meinitzer, A., Mangge, H., Kriegshäuser, G., Halwachs-Baumann, G., Reininghaus, E. Z., Bengesser, S. A., & Schnedl, W. J. (2016). Canadian journal of gastroenterology & hepatology.

**2017 – Circadian profiling reveals higher histamine plasma levels and lower diamine oxidase serum activities in 24% of patients with suspected histamine intolerance compared to food allergy and controls.** Pinzer, T. C., Tietz, E., Waldmann, E., Schink, M., Neurath, M. F., & Zopf, Y. *Allergy*. **2017**; 73(4), 949–957

**2016 – Histamine intolerance and dietary management: A complete review I.** San Mauro Martin\*, S. Brachero, E. Garicano Vilar **2016** Aug 30. *Allergol Immunopathol (Madr)*.

**2015 – Histamine, histamine intoxication and intolerance.** E. Kovacova-Hanuszkova, T. Buday, S. Gavliakova, J. Plevkova\*Comenius University in Bratislava, Jessenius Faculty of Medicine, Department of Pathophysiology, **2015** Sep-Oct *Allergol Immunopathol*

**2014 – Evaluation of the inhibitory effect of various drugs/active ingredients on the activity of human diamine oxidase in vitro.** Leitner, R.; Zoernpfenning, E.; Missbichler, A. *Clin. Transl. Allergy* **2014**, 4, P23.

**2010 - Histamine intolerance: a metabolic disease?** Schwelberger H. G. (2010). *Inflammation research: official journal of the European Histamine Research Society*.

**2007 – Histamine and histamine intolerance.** Laura Maintz and Natalija Novak. Department of Dermatology and Allergology, University of Bonn, Germany. **2007** – *The American Journal of Clinical nutrition*

**2001 – Human kidney diamine oxidase: heterologous expression, purification, and characterization.** Bradley O. Elmore, John A. Bollinger, David M. Dooley. **2001**. *J Biol Inorg Chem* (2002) 7: 565-579

## **8. Diamine Oxidase Activity**

**2023 - Advances in the Clinical Application of Histamine and Diamine Oxidase (DAO) Activity: A Review.** Tan Z, Ou Y, Cai W, Zheng Y, Li H, Mao Y, Zhou S, Tu J. **2023**. *Catalysts*.

**2022 - Basal Serum Diamine Oxidase Levels as a Biomarker of Histamine Intolerance: A Retrospective Cohort Study.** Cucca V, Ramirez GA, Pignatti P, Asperti C, Russo M, Della-Torre E, Breda D, Burastero SE, Dagna L, Yacoub MR. *Nutrients*. 2022.

**2020 – Standardization of a colorimetric technique for determination of enzymatic activity of diamine oxidase (DAO) and its application in patients with clinical diagnosis of histamine intolerance.** Camila Beltrán-Ortiz, Teresa Peralta, Verónica Ramos, Magdalena Durán, Carolina Behrens, Daniella Maureira, Maria A. Guzmán, Carla Bastias and Pablo Ferrer. *World Allergy Organization Journal*. **2020**. 13:100457

**2019 – In vitro determination of diamine oxidase activity in food matrices by an enzymatic assay coupled to UHPLC-FL.** Comas-Basté, O.; Latorre-Moratalla, M.L.; Sánchez-Pérez, S.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. *Anal. Bioanal. Chem*. **2019**, 411, 7595–7602.

**2019 – Analytical possibilities of Putrescine and Cadaverine enzymatic colorimetric determination in tuna based on diamine oxidase: A critical study of the use of ABTS.** Jesús Navarro, Isabel Sanz-Vicente, Rebeca Lozano, Susana de Marcos, Javier Galbán. *Elsevier-Talanta*. **2019**.

**2018 - Mapping of the binding sites of human diamine oxidase (DAO) monoclonal antibodies.** Schwelberger HG, Feurle J, Houen G. *Inflamm Res.* 2018

**2018 - Serum levels of histamine and diamine oxidase in multiple sclerosis.** Rafiee Zadeh, A., Falahatian, M., & Alsahebhosoul, F. 2018. *American journal of clinical and experimental immunology.*

**2017 – New approach for the diagnosis of histamine intolerance based on the determination of histamine and methylhistamine in urine.** Comas-Basté, O.; Latorre-Moratalla, M.L.; Bernacchia, R.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. *J. Pharm. Biomed. Anal.* **2017**, 145, 379–385.

**2016 – Serum diamine oxidase activity in patients with histamine intolerance.** G Manzotti, D Breda, M Di Giacchino and SE Burastero, **2016**, Vol. 29(1) 105-111 *International Journal of Immunopathology and Pharmacology.*

**2014 - Role of histamine and diamine oxidase enzyme in Multiple Sclerosis.** Multiple sclerosis and related disorders. Farrokhi, Mehrdad & Etemadifar, Masoud & Rezaei, Ali & Amani Beni, Ali & Jahanbani, Hamidreza. (2014).

**2013 – Serum diamine oxidase activity as a diagnostic test for histamine intolerance.** Ema Mušič, Peter Korošec, Mira Šilar, Katja Adamič, Mitja Košnik, Matija Rijavec. **2013** *Wien Klin Wochenschr* (2013) 125:239–243.

**2012 - The diamine oxidase gene is associated with hypersensitivity response to non-steroidal anti-inflammatory drugs.** Agúndez, J. A., Ayuso, P., Cornejo-García, J. A., Blanca, M., Torres, M. J., Doña, I., Salas, M., Blanca-López, N., Canto, G., Rondon, C., Campo, P., Laguna, J. J., Fernández, J., Martínez, C., & García-Martín, E. (2012). *PloS one.*

## **9. Diamine Oxidase Supplements**

**2020 – Lyophilised legume sprouts as a functional ingredient for diamine oxidase enzyme supplementation in histamine intolerance.** Oriol Comas-Basté, M. Luz Latorre-Moratalla, Judit Rabell-González, M Teresa Veciana-Nogués, M. Carmen Vidal-Carou. *Food Science and Technology* 125. **2020**. 109201

**2020 – DAOSIN: Evaluation of porcine diamine oxidase for the conversion of histamine in food-relevant amounts.** Lucas Kettner, Ines Seitzl, and Lutz Fischer. *Journal of Food Science.* **2020**.

**2018 - Biogenic Amines in Plant-Origin Foods: Are They Frequently Underestimated in Low-Histamine Diets?** Sánchez-Pérez S, Comas-Basté O, Rabell-González J, Veciana-Nogués MT, Latorre-Moratalla ML, Vidal-Carou MC. 2018. *Foods.*

**2019 – Diamine oxidase supplementation improves symptoms in patients with histamine intolerance.** Wolfgang J. Schnedl, Michael Schenk, Sonja Lackner, Dietmar Enko, Harald Mangge, Rorlan Forster. *Food Sci Biotechnol.* **2019**. 1779-1784

**2018 – Diamine Oxidase Supplementation in Chronic Spontaneous Urticaria: A Randomized, Double-Blind Placebo-Controlled Study.** Mona-Rita Yacoub, Giuseppe A. Ramirez, Alvise Berti, Giuseppe Mercurio, Daniela Breda, Nicoletta Saporiti, Samuele Burastero, Lorenzo Dagna, Giselda Colombo. *Int Arch Allergy Immunol.* **2018**.

## 10. PhD Thesis and ongoing doctoral programs

**Ongoing - Relationship between histamine intolerance symptoms with DAO enzyme polymorphisms.** Doctoral thesis by Adriana Duelo.

**2020 – Intolerància a la histamina. Noves estratègies de diagnòstic i tractament.** Tesi doctoral d'Oriol Comas-Basté. Directors: M. Carmen Vidal-Carou, and M. Luz Latorre-Moratella.

**2020** October – Universitat de Barcelona, Barcelona, Spain

**2017 – Diamine Oxidase activity in patients with migraine and the effect of enzymatic supplementation as a preventive treatment for episodic migraine.** Doctoral Thesis of Izquierdo-Casas, J. Directors: Soler, L, Lorente, M. **2017** October – International University of Catalonia, Barcelona, Spain

**2016 – Aspectos clínicos y nutricionales de la intolerancia a la histamina en pacientes pediátricos con sintomatología digestiva crónica.** Doctoral tesis by Rosell Camps, Antonio. Directors: Palou Oliver, Andreu; Picó Segura, Catalina. **2016** February – Universitat de les Illes Balears, Spain

## 11. Posters and Awards

- Posters 2022:** Dysbiosis of intestinal microbiota in patients with histamine intolerance.
- Posters 2019:** Putrescine and cadaverine reduce the degradation rate of histamine by DAO enzyme.
- Posters 2019:** Determination of histamine-degrading activity in food matrices by an enzymatic assay coupled to UHPLC-FL
- Posters 2019:** Histamine-degrading capacity of lyophilised legume sprouts.
- Posters 2018:** What about legumes as a plant source of the DAO enzyme?
- Posters 2018:** Low-histamine diet supplemented with exogenous diamine oxidase enzyme is useful for treating migraine in patients with DAO deficiency
- Posters 2016:** Serum DiAmine Oxidase (DAO) activity in migraine patients
- Posters 2013:** Dietary treatment and blood DiAmine Oxidase (DAO) values in cyclic vomiting syndrome (cvs)
- Posters 2013:** Dietary treatment and blood DiAmine Oxidase (DAO) values in patients affected vomiting and other gastrointestinal and neurological symptoms
- Posters 2013:** Diamine Oxidase deficiency as a predisposing factor for migraine
- Awards 2012:** Second prize – Catalan Society of Neurology
- Posters 2010:** Evaluation of DiAmine Oxidase deficiency in patients with migraine

## **12. Ongoing research projects**

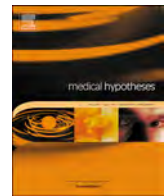
1. Association between genetic DAO deficiency, CGRP plasmatic concentration, Histamine accumulation and migraine symptoms.
2. Publication of medical hypothesis. Prevalence of genetic DAO deficiency in ADHD patients.
3. Effect of DAO supplementation in ADHD patients with DAO deficiency.
4. Prevalence of genetic DAO deficiency in fibromyalgia patients.
5. Effect of DAO supplementation in patients with fibromyalgia.
6. Effect of pharmaceutical drugs on DAO activity.
7. Effect of DAO supplementation on different groups of symptoms associated with histamine accumulation.
8. Reversion of alcohol-driven DAO inhibition through DAO + yeasts supplementation.
9. Prevalence of DAO deficiency in people with sleep disorders.
10. Effect of DAO supplementation on patients with sleep disorders.
11. Effect of DAO supplementation / topical application on dermatological lesions of cats and dogs.
12. Application of the supercritical CO<sub>2</sub> as an antimicrobial agent in the extraction process of the Diamine Oxidase.

## **13. Published data**

Attached at this document are the copies of the scientific papers and posters mentioned above.

# **Diamine Oxidase and Migraine**

**Associations between migraine, celiac disease, non-celiac  
gluten sensitivity and activity of diamine oxidase. K.  
Griauzdaitė, K. Maselis, A. Žvirblienė, A. Vaitkus, D. Jančiauskas,  
I. Banaitytė-Baleišienė, L. Kupčinskas, D. Rastenytė. Medical  
Hypotheses 142. 2020. 109738**



## Associations between migraine, celiac disease, non-celiac gluten sensitivity and activity of diamine oxidase

K. Griauzdaitė, K. Maselis\*, A. Žvirblienė, A. Vaitkus, D. Jančiauskas, I. Banaitytė-Baleišienė, L. Kupčinskas, D. Rastenytė

Hospital of Lithuanian University of Health Sciences Kauno Klinikos, Eivenių g. 2, Kaunas, Lithuania

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### ABSTRACT

**Background and pilot study:** Recent reports reveal a close relationship between migraine and gastrointestinal disorders (GI), such as celiac disease (CD) and non-celiac gluten sensitivity (NCGS). CD is a genetic autoimmune disorder, which affects the mucosa of the small intestine. Gluten, found in various grains, not only plays a major role in the pathophysiology of CD and NCGS, but also aggravates migraine attacks. Another common food component, which can induce migraine headaches, is histamine. Diamine oxidase (DAO) is an enzyme, which degrades histamine. Reduced activity of DAO means reduced histamine degradation, which can cause histamine build-up and lead to various symptoms, including headaches and migraine. In this paper we propose a hypothesis, that in pathogenesis of migraine, low serum DAO activity is related to CD and NCGS. We also conducted our own pilot study of 44 patients with severe migraine in efforts to evaluate the co-presence of decreased serum DAO activity and celiac disease/NCGS in patients. 44 consecutive migraine patients were divided into 2 groups: decreased DAO activity (group 1; n = 26) and normal DAO activity (group 2; n = 18). All patients were screened for celiac disease. The diagnosis of NCGS was made after exclusion of CD, food allergies and other GI disorders in the presence of gluten sensitivity symptoms. Furthermore, dietary recommendations were given to all participants and their effects were assessed 3 months after the initial evaluation via the MIDAS (Migraine Disability Assessment) questionnaire.

**Results and conclusions:** Only 1 patient fit the criteria for celiac disease, rendering this result inconclusive. Pathological findings of the remainder of patients were attributed to NCGS (n = 10). 9 of 10 patients with NCGS belonged to the decreased serum DAO activity group (group 1; n = 26), suggesting a strong relationship between reduced serum DAO activity and NCGS. MIDAS questionnaire revealed, that patients with decreased serum DAO activity were more severely impacted by migraine than those with normal DAO activity, and this remained so after our interventions. Dietary adjustments significantly reduced the impact of migraine on patients' daily activities after 3 months in both groups. We argue, that migraine, celiac disease and NCGS may benefit from treatment with a multidisciplinary approach, involving neurologists, gastroenterologists and dietitians.

### Introduction

Migraine is a common headache disorder with prevalence of approximately 12% of total population [1]. It can have a severe impact on the quality of life of an individual by affecting personal life, social life and work performance, while the treatment costs create a considerable economical burden for the healthcare system [2].

Recent reports reveal a close relationship between migraine and gastrointestinal (GI) disorders [3]. It is becoming evident that proper treatment of underlying GI conditions, such as celiac disease, reduces the severity and frequency of migraine headaches [4]. Celiac disease is

a genetic autoimmune disorder, which affects the mucosa of the small intestine [5]. Gliadin, a component of gluten, is key to eliciting a damaging immune response, hence observed morphological changes can be controlled via a gluten-free diet [6]. Diagnosis of CD requires serological, histological and genetic confirmation [5]. If patients do not meet the diagnostic criteria of CD, but experience gluten sensitivity, non-celiac gluten sensitivity (NCGS) can be diagnosed, provided food allergies, CD and other GI diseases are ruled out [7].

Diamine oxidase (DAO) is an enzyme, which degrades histamine. Reduced concentrations or activity of DAO can lead to elevation in histamine concentration, leading to allergic reactions, headaches and

\* Corresponding author at: Baltijos g. 68-25, Kaunas, Lithuania.

E-mail address: [kipras.maselis@gmail.com](mailto:kipras.maselis@gmail.com) (K. Maselis).

migraine [8–13]. Furthermore, histamine intolerance has been shown to closely resemble responses elicited by gluten, which indicates, that a close relationship between histamine and gluten may be present [14].

### The hypothesis

Our hypothesis is that migraine, alongside decreased serum DAO concentration and sensitivity to histamine, is closely related to gluten sensitivity (celiac disease and NCGS). The hypothesis stems from epidemiological and pathophysiological evidence, presented below.

### Supporting evidence

#### *Central and enteric nervous systems*

Common pathophysiological ground for gastrointestinal and neurological diseases can be established by appreciating the relationship between the enteric nervous system (ENS) and central nervous system (CNS). The brain-gut axis idea has been proposed, suggesting that information is relayed and integrated both from the CNS to ENS, and from ENS to CNS [15]. As a result, the possible connections between GI syndromes and neurological syndromes can be explored and would suggest that CNS-ENS interaction may be pathophysiologically significant for co-occurrence of diseases, such as migraine and celiac disease [16].

#### *Migraine, celiac disease and NCGS*

A number of epidemiological studies have found a close relationship between migraine and gastrointestinal disorders, such as celiac disease and NCGS. A case-control study of 502 patients with celiac disease and NCGS demonstrates a bigger prevalence of migraine compared to the general population [17], while another case-control study of 90 migraine patients reveals a reverse association, showing increased celiac disease prevalence in patients with migraine compared to the general population [18].

Such associations are grounded in a growing body of evidence, which suggests that the diseases have extensive pathophysiological links. On a molecular level, it has been implied that toxic gluten peptides, which induce a damaging immune response to the small intestine in celiac disease, are a product of an extracellular enzyme transglutaminase 2 (TG2) [19]. Increased TG2 expression results in more toxic gluten peptides, eliciting a more severe immune response. TG2 expression is upregulated by tumor necrosis factor alpha (TNF $\alpha$ ) through p38 MAPK (mitogen-activated protein kinase) pathway [20]. However, activation of MAPK pathways not only increases TG2 expression, but also upregulates calcitonin gene-related peptide (CGRP) synthesis and release [21], which has been closely associated with migraine [22]. Therefore, celiac disease can be presumed to be associated with migraine on a molecular level.

#### *Migraine and gluten*

Gluten has been demonstrated to have damaging effects on the nervous tissue in patients with celiac disease and NCGS. The effects can range from cerebellar ataxia to depression and migraine [23]. More importantly, such syndromes can occur in well-nourished patients, excluding malnutrition as an aetiological factor. In a prospective study of 215 patients with axonal neuropathy, 34% had positive IgA antigliadin antibodies, compared to 12% in the healthy control group, revealing increased risk of both gluten sensitivity and celiac disease in patients with peripheral neuropathy, compared to the general population [24]. In order to analyze the actual effects on gluten itself, a number of studies prescribed gluten-free diets to patients with migraine, which had statistically positive results on the frequency and severity of migraine attacks [4,18].

### *Migraine, serum DAO activity and histamine*

Histamine is a biogenic amine, which can initiate and aggravate migraine attacks [25]. It is present in many foods, and some migraineurs tend to avoid foods high in histamine, such as certain fish, cheeses, processed meat, fermented foods and alcoholic beverages, presumably due to them provoking migraine attacks [26]. Histamine is metabolised to inactive components by diamine oxidase (DAO). DAO is released into GI tract lymphatically, which is a response to increased histamine release. It has been hypothesized that secretion of DAO has a purpose to control the unwanted effects of histamine [27]. Therefore, it is rational to presume, that decreased DAO activity would imply hindered ability to degrade histamine and greater subsequent unwanted effects of histamine release. Izquierdo-Casas et al. (2018) found that decreased serum DAO activity was more closely associated with migraine headaches rather than non-migraine headaches [28], and their subsequently conducted randomized controlled trial of 100 patients with migraine and decreased DAO activity (2019) have found, that administering DAO supplements in these patients significantly reduced the duration of migraine attacks by 1.4 h ( $p = 0,0217$ ), while the placebo group did not experience significant change (0,9 h) [29]. Furthermore, García-Martín et al. (2015) discovered certain genes, responsible for decreased DAO activity, and that patients with such genes were significantly more likely to develop migraine [12].

#### *Migraine, DAO activity and celiac disease/non-celiac gluten sensitivity – our findings*

The information regarding the link between serum DAO activity and mentioned gastrointestinal disorders is scarce. To address the issue we conducted our own pilot study in efforts to evaluate the co-presence of decreased serum DAO activity and celiac disease/NCGS in patients with severe migraine. Furthermore, we gave general dietary recommendations regarding gluten sensitivity to all participants and assessed its effects 3 months after the initial evaluation.

### Pilot study

#### *Objectives*

1. To evaluate the correlation between decreased DAO activity and the prevalence of celiac disease and NCGS in patients with severe migraine.
2. To evaluate the impact of migraine on patients' daily activities before investigation and 3 months post-investigation.

#### *Methods*

59 consecutive patients with severe migraine, treated in Hospital of Lithuanian University of Health Sciences Kauno klinikos from December 2015 to December 2016, presenting with symptoms related to celiac disease or non-celiac gluten sensitivity (NCGS) were considered in the study. Afterwards, 15 patients were excluded (other cause of dyspepsia identified – 2 patients; lifestyle considered to be too hectic to follow administered treatment – 2 patients; patients not following administered migraine treatment with previously diagnosed migraine – 4 patients; consent not given – 7 patients). As a result, 44 patients (5 male, 39 female, aged 18+, mean age 41,31  $\pm$  95% CI 37,93–44,70) were enrolled in the study. Severe migraine was defined as experiencing 4 or more migraine attacks in a month and/or having drug-resistant migraine. Diamine oxidase (DAO) activity in serum was tested for all patients. Patients were divided into 2 groups: decreased DAO activity (group 1;  $n = 26$ ) and normal DAO activity (group 2;  $n = 18$ ). Prevalence of celiac disease and NCGS were evaluated with tissue transglutaminase antibody titer in serum (anti-tTG) and histological examination of duodenum mucosa. Anti-tTG was tested by ELISA

**Table 1**  
Comparison of anti-tTG titers between groups 1 and 2.

Characteristics	Group 1, n (%) (Decreased DAO activity)	Group 2, n (%) (Normal DAO activity)	p
Participant count	n = 26	n = 18	$\chi^2 = 2,23$ ; df = 1; p = 0,14
anti-tTg-IgA concentration:	–	–	
Normal value (< 12 U/ml)	23 (88,46)	18 (100,00)	
Positive findings (> 18 U/ml)	3 (11,54)	0 (00,00)	

and histologic samples were obtained by endoscopic biopsy. Histologic findings were interpreted in accordance with the Marsh classification. The impact of migraine on daily activities was evaluated using Migraine Disability Assessment (MIDAS) on first admission and again in 3 months, after the patients had received dietary recommendations. All data were stored using Excel 2007, and statistical significance of results was tested with SPSS 2.0. Descriptive statistics methods were used to calculate average age and other mean values. Chi-square test and Fisher's exact test were used for categorical variables (Chi-square was used for larger samples, whereas Fisher's exact test was used when samples are small), whereas *t*-test was used to evaluate power for continuous variables;  $p < 0,05$  was held significant in all tests.

## Results

### 1. Association of DAO activity with celiac disease and NCGS.

#### Anti-tTG findings

In group 1, 3 of 26 patients had positive findings (18 U/ml), whereas in group 2 no patients had abnormal results ( $\chi^2 = 2,23$ ; df = 1; p = 0,14). Results are presented in Table 1.

#### Histologic findings

In group 1, 8 of 26 patients displayed abnormal duodenum mucosa (7 – Marsh I; 1 – Marsh IIIA), whereas in group 2 only 1 patient (Marsh IIIA). Results are presented in Table 2.

#### Combined data

According to clinical, laboratory and histologic findings, only 1 patient fit the criteria for celiac disease. The pathological findings of the remainder of patients were attributed to NCGS. NCGS prevalence in both groups are presented in Table 3.

### 2. Evaluation of impact of migraine on patients' daily activities before investigation and 3 months post-investigation.

The evaluation was carried out via the MIDAS questionnaire. The first evaluation occurred during the first consult with a neurologist. The second evaluation took place after 3 months, after the patient had already had a consult with a gastroenterologist and had been informed about general dietary recommendations regarding gluten sensitivity and histamine intolerance. Testing was made for both groups 1 and 2.

Patients in group 1 (decreased DAO activity) showed statistically lower average of days, during which patients' daily activities were impaired (Total: 85,54 CI [61,54–109,54] on first admission, and 71,04 CI [49,70–92,37] after 3 months, a decrease of 14,50,  $p = 0,05$ ), as

**Table 2**  
Comparison of duodenum histological findings between groups 1 and 2.

Characteristics	Group 1, n (%) (Decreased DAO activity)	Group 2, n (%) (Normal DAO activity)	p
Participant count	n = 26	n = 18	$\chi^2 = 5,76$ ; df = 2; p = 0,06
Histology, in accordance with Marsh:	–	–	
Normal duodenum mucosa	18 (69,23)	17 (94,44)	
Marsh I	7 (26,92)	0 (00,00)	
Marsh IIIA	1 (3,85)	1 (5,56)	

**Table 3**  
Comparison of prevalence of NCGS between groups 1 and 2.

Characteristics	Group 1, n (%) (Decreased DAO activity)	Group 2, n (%) (Normal DAO activity)	p
Participant count	n = 26	n = 18	$\chi^2 = 5,43$ ; df = 1; p = 0,02
Prevalence:	–	–	
Disease-free	16 (61,54)	17 (94,44)	
Non-celiac gluten sensitivity	9 (34,62)	1 (5,55)	

well as slightly decreased pain severity during headaches (7,50 CI [7,03–7,97] on first admission, and 7,15 CI [6,75–7,56] after 3 months, a decrease of 0,34,  $p = 0,004$ ).

Patients in group 2 (normal DAO activity) also experienced statistically lower average of days, during which patients' daily activities were impaired (Total: 48,06 CI [38,89–57,22] on first admission, and 38,89 CI [31,64–46,14] after 3 months, a decrease of 9,17 days on average,  $p = 0,003$ ), as well as slightly decreased pain severity during headaches (7,11 CI [6,70–7,52] on first admission, and 6,61 CI [6,12–7,10] after 3 months, a decrease of 0,50,  $p = 0,02$ ).

When comparing the average number of days with migraine headache in both groups prior to our interventions, *t*-test reveals, that the group 1 (decreased DAO activity) is much more affected than group 2 (85,54 days vs. 48,06 days, respectively,  $p = 0,0001$ ). This remains true after 3 months (71,04 days vs. 38,89 days, respectively,  $p = 0,0001$ ).

## Discussion

In our study, only 1 migraine patient fully fit the criteria for the diagnosis of celiac disease, therefore, no clear statements can be made about the difference in the prevalence of celiac disease between patients with different DAO activity. A shortage of the total number of enrolled participants may have led to this result.

However, 9 out of 10 migraine patients diagnosed with NCGS were found to have decreased DAO activity, suggesting a positive association between migraine, NCGS and serum DAO activity and supporting our hypothesis. The causal relationship and the nature of the associations remain unclear and many models of explanation may fit. From a clinical perspective, the current results suggest, that in patients with gluten sensitivity (provided CD, food allergies and other GI diseases are ruled out), it would be rational to test serum DAO activity and consider addressing migraine treatment through dietary changes, such as reducing gluten and histamine content in foods. Therefore, gluten sensitivity and response to diet in migraine patients may prove to be a useful screening

tool.

The evaluation of impact of migraine on patients' daily activities before investigation and 3 months post-investigation revealed, that the quality of life in migraine patients, who have decreased serum DAO activity, is more severely impacted than in those with normal DAO activity, and this remains true after treatment. Therefore it seems, that DAO activity is related to migraine severity. Luckily, results demonstrated a noteworthy reduction in the number of days, during which patients' daily activities were impaired; as well as slightly decreased pain severity during headaches in both groups. The changes were likely to be caused by patients' dietary adjustments due to the second evaluation taking place after the patients had already had a consult with a gastroenterologist and dietitian and had been informed about the dietary recommendations regarding gluten sensitivity and histamine intolerance. These results support the hypothesis, that migraine, gluten sensitivity, and reduced serum DAO activity are related and also suggest, that in patients with gluten sensitivity and reduced DAO activity, diet can be a major treatment factor.

Since migraine and GI disorders have been found to be closely related [3], we recommend to treat migraine, celiac disease, and NCGS with a multidisciplinary approach, involving neurologists, gastroenterologists and dietitians. We hope this information will help to improve the treatment of severe migraine, gastrointestinal disorders, and the pain that accompanies these disorders. Earlier and more accurate identification of comorbid conditions can reduce the frequency of migraine headaches, reduce treatment costs, and improve the quality of patients' lives.

Although our pilot study supports the hypothesis, it does not prove it by far. The study itself is limited by a wide array of factors, such as inferior study design and small sample size, which do not allow to draw decisive conclusions. Furthermore, although the data about patients' comorbid conditions and previously used medications were obtained at the time, information was not documented. Also, even though we gave patients general recommendations regarding histamine and gluten containing products, strict and consistent control over their diet was not maintained, therefore, it cannot be concluded, that diet was the main factor in observed improvements. All of the mentioned limitations may create bias and subsequently, inaccurate results, therefore, further research on the topic is warranted.

### Conflict of interest

Authors disclose, that there are no financial or personal relationships with other people or organisations that could inappropriately influence their work.

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**Association of Diamine oxidase (DAO) variants with the risk for migraine from North Indian population.** Sukhvinder Kaura, Arif Alib, Yaser Siahbalaec, Uzair Ahmadc, Fazila Nargisc, A.K. Pandeyd, Balkirat. **2019** October 21-25400- / © 2019 Elsevier B.V.



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## Association of Diamine oxidase (DAO) variants with the risk for migraine from North Indian population

Sukhvinder Kaur<sup>a,\*</sup>, Arif Ali<sup>b</sup>, Yaser Siahbalaie<sup>c</sup>, Uzair Ahmad<sup>c</sup>, Fazila Nargis<sup>c</sup>, A.K. Pandey<sup>d</sup>, Balkirat Singh<sup>e</sup>

<sup>a</sup> UGC-PDF, Gene Expression Lab, Department of Biosciences, Jamia Millia Islamia, New Delhi, India

<sup>b</sup> UGC-BSR-FF, Department of Biosciences, Jamia Millia Islamia, New Delhi, India

<sup>c</sup> Department of Biosciences, Jamia Millia Islamia, New Delhi, India

<sup>d</sup> Head, Department of Physiology, ESIC Medical College & Hospital, Faridabad, India

<sup>e</sup> NC Medical College & Hospital, Panipat, India

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### ABSTRACT

**Background:** Migraine is a common neurovascular disorder affected by various levels of neurotransmitters. Low histamine metabolism is also related with pathophysiology of migraine. As diamine oxidase (DAO) gene variants are linked with higher levels of histamine in migraine patients, we investigated the possible relationship of two variants rs2052129 and rs10156191 of this gene with migraine risk in North Indian population.

**Methods:** A case-control study for 250 migraine patients and 250 matched healthy controls was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

**Results:** We found statistically significant differences in allelic frequencies of rs2052129 ( $p = .009$ , OR = 1.462; 95% CI: 1.098–1.947) and rs10156191 ( $p = .019$ , OR = 1.430; 95% CI: 1.060–1.928) variants in DAO gene. For rs10156191, we were able to show statistically significant association at all genotypic, dominant and allelic levels in both MA (for T allele,  $p = .020$ ; OR = 1.662, 95% CI: 1.083–2.551) as well as in female subgroup (for T allele,  $p = .025$ , OR = 1.460; 95% CI: 1.049–2.033). But no such significant association was found in clinical sub grouping of migraine in rs2052129 as  $p > .05$ . However in gender analysis, protective effect of T allele in male migraine patients for rs2052129 (OR < 1) was found.

**Conclusions:** Our findings clearly indicated that female patient with rs10156191T allele and in MA subgroup showed an increased risk for migraine. Our data also indicated that rs2052129T variant showed a significant role in migraine susceptibility of this population.

### 1. Introduction

Migraine is a multi-factorial disorder having a prevalence of approximately 12% with a female: male ratio of 2–3:1 (Lipton et al., 2007; De Vries et al., 2006). Finding genes for different forms of common migraine (with and without aura) and their pathogenic roles in defining this disorder have not gained much success yet. Candidate gene association and genome-wide association studies have been used to reveal the possible relation among various SNPs and migraine risk (Anttila et al., 2013; Chasman et al., 2011). These studies are mostly related with neurotransmitters including serotonergic and dopaminergic systems and have inconsistent and inconclusive outcomes (Freilinger et al.,

2012). Even interethnic variability occurs in these association studies as some genes which were associated with Indian population (Ghosh et al., 2013) showed negative results with that of Chinese (An et al., 2013; Fan et al., 2014), Swedish (Ran et al., 2014) or Spanish (Sintas et al., 2015).

Apart from neurotransmitters like serotonin and dopamine, histamine might have a significant role in pathogenesis of migraine (Alstadhaug, 2014). It has been reported that histamine-rich foods may trigger migraine attacks. In migraine patients, histamine concentrations in plasma are reported to be increased during headache attacks and for symptom-free periods as compared with controls as ( $p < .001$ ) (Maintz and Novak, 2007). Histamine N-methyltransferase (HNMT) and Diamine oxidase (DAO) or amiloride binding protein 1 (ABP1) are the two

**Abbreviations:** DAO, Diamine oxidase; PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism; SNP, Single nucleotide polymorphism; HNMT, Histamine N-methyltransferase; ABP1, Amiloridebinding protein 1; MO, Migraine without aura; MA, Migraine with aura; OPD, Outpatient department; HWE, Hardy-Weinberg equilibrium; OR, Odds ratios; CI, Confidence intervals; SD, Standard deviation

\* Corresponding author at: 309, Gene Expression Laboratory, Department of Biosciences, Jamia Millia Islamia, New Delhi, India.

E-mail address: [singhrp12@rediffmail.com](mailto:singhrp12@rediffmail.com) (S. Kaur).

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enzymes, responsible for degradation of histamine whereas histidine decarboxylase enzyme can synthesize it by decarboxylation from its precursor histidine. *HNMT* is related with degradation of intracellular histamine whereas *DAO* is mainly for scavenging extracellular one after its release (Preuss et al., 1998; Wang et al., 2002). *DAO* enzyme has three common non-synonymous single nucleotide polymorphisms (SNPs) that are: rs10156191 (Thr16Met), rs1049742 (Ser332Phe) and rs1049793 (His645Asp) which can reduce protein's activity by altering its form (Ayuso et al., 2007; Garcia-Martin et al., 2007). Another SNP rs2052129 (G4586 T), present in promoter region of gene, has been related with decreased transcriptional activity of *DAO* (Maintz et al., 2011). Various studies have reported that the patients with minor alleles of rs1049793, rs10156191 and rs2052129 SNPs have lower serum *DAO* activity on comparison with controls (Garcia-Martin et al., 2015).

The purpose of this study is to find a link between two functional SNPs i.e. rs2052129 and rs10156191 (related with reduced *DAO* activity) with migraine risk in North Indian population. This is the first case-control association study to investigate the role of these variants in common migraine patients from this population.

## 2. Methods

### 2.1. Subjects

We studied 250 migraine patients [55 males (22%) and 195 females (78%)], and excluded other headache types, according to the guidelines recommended by International Classification of Headache disorders, 3rd edition (International Headache Society, 2013) and 250 controls [65 males (26%) and 185 females (74%)]. Migraineurs were recruited from those who visited OPD of ESIC medical College & Hospital, Faridabad, India. Migraine patients were diagnosed as having either migraine with aura (MA) or migraine without aura (MO). Controls were healthy volunteers matched for sex and age with patients who did not suffer from migraine and any other types of headaches. A written informed consent from all the subjects was taken prior to this study and it was further cleared by different Ethics Committees of JMI, New Delhi and that of ESIC Medical College & Hospital, Faridabad. These subjects were also taken in other case-control studies conducted by our group (Kaur et al., 2018, 2019).

### 2.2. Genotyping

Genotyping was performed in genomic DNA isolated by both salting out method (Miller et al., 1988) as well as from whole genomic DNA extraction kit (from Bangalore Genei) after collecting 5 ml venous blood in EDTA vials from subjects. Polymorphisms were checked via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to detect SNPs. Self designed primers used for rs10156191 were:

Sense primer- 5'ATTCCATGGCCCTAACCTGAG 3'.

Antisense primer-5'GTGCACTGCCTTCAGCTCTT 3'.

Whereas for rs2052129 were as follows:

Sense primer-5'GCCAGGGTAGCTAAGGCTATG 3'.

Antisense primer-5' ACTCCTGAGCCACGAACTTTT 3'.

Cycling conditions for this study were: An initial denaturation was done for 5 min at 95 °C, then there were 35 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for rs2052129 and 56 °C for rs10156191 for 30 s respectively, and extension at 72 °C for 90 s followed by a final extension at 72 °C for 7 min. Restriction enzymes used in RFLP for rs2052129 and rs10156191 were *DrdI* and *MscI* (from New England, Biolabs) respectively. PCR products of 188 bp for rs2052129 were digested into 98 bp and 90 bp for minor allele A by *DrdI* enzyme and 228 bp PCR products of rs10156191 were divided into 142 bp and 86 bp fragments for minor allele T by *MscI* at 37 °C. The outcomes of PCR followed by restriction analysis of these SNPs are shown in Figs. 1-4.

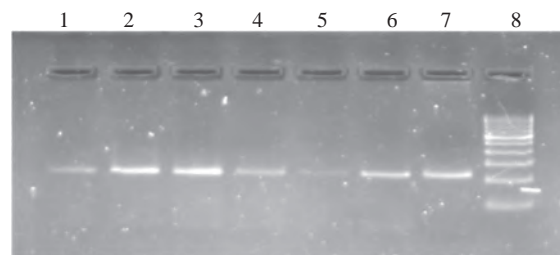


Fig. 1. Lane: 1–7 showing PCR products of 228 bp for rs10156191SNP. Lane: 8-100 bp DNA ladder.

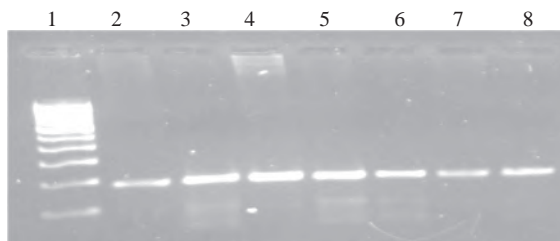


Fig. 2. Lane: 2–8 showing PCR products of 188 bp for rs2052129 SNP. Lane: 1-100 bp DNA ladder.

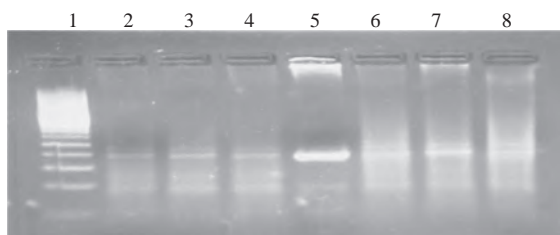


Fig. 3. Lane-2-8 showing GT (3 fragments: 188 bp, 98 bp and 90 bp) of rs2052129 SNP. Lane-5-showing GG (1fragment of 188 bp). Lane-1 50 bp DNA ladder.

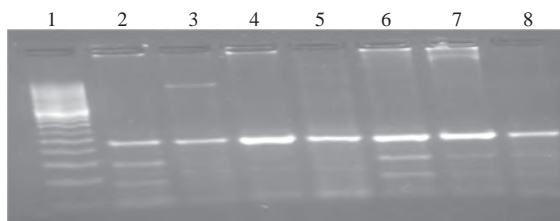


Fig. 4. Lane-2, 6, 7 and 8 showing CT (3 fragments: 228 bp, 142 bp and 86 bp) of rs10156191 SNP. Lane-3, 4 and 5 showing CC (1 fragment: 228 bp). Lane-1 50 bp DNA ladder.

### 2.3. Statistical analysis

Hardy-Weinberg equilibrium (HWE) was confirmed for subjects using Chi-square goodness of fit test. Statistical analysis was performed using SPSS 24.0 and  $p < .05$  was taken statistically significant for this data. The risk of migraine was taken as odds ratios (OR) for mutant allele carriers with 95% confidence intervals (CI) between groups. Comparison of allelic and genotypic frequencies was done by Chi-squared test. The categorical variables were represented by numbers and percentages however continuous variables were shown as means  $\pm$  standard deviation (SD) for this test. Power analysis was done using Quanto 1.2.4. The SNPs were to show a twofold increase in risk of

**Table 1**  
Genotypic and allelic distribution of rs2052129 polymorphism in this study.

	Genotypic distribution N (%)			Allelic distribution N (%)	
	GG	GT	TT	G	T
Migraine(250)	132(52.8)	90(36)	28(11.2)	354(70.8)	146(29.2)
HC(250)	148(59.2)	94(37.6)	8(3.2)	390(78)	110(22)
MO(180)	96(53.3)	70(38.9)	14(7.8)	262(72.8)	98(27.2)
MA(70)	38(54.3)	24(34.3)	8(11.4)	100(71.4)	40(28.6)
Male migraine (55)	42(76.4)	9(16.4)	4(7.2)	93(84.5)	17(15.5)
Female migraine (195)	104(53.3)	86(44.1)	5(2.6)	294(75.4)	96(24.6)
Male control (65)	33(50.8)	26(40)	6(9.2)	92(70.8)	38(29.2)
Female control (185)	98(53)	72(38.9)	15(8.1)	268(72.4)	102(27.6)

migraine with 250 case-controls used here with significance level set at 0.05. The prevalence of migraine was taken as 0.12. By assuming the minor allele frequencies as 22% for rs2052129 and 19.4% for rs10156191 in control groups we achieved 45.3 and 37.4% power for rs2052129 and rs10156191 respectively.

### 3. Results

This study included a cohort of 250 migraine patients (70 MA and 180 MO) and 250 healthy controls. The mean age of patients was  $34.55 \pm 6.699$  years and that of controls was  $35.40 \pm 6.123$  years. All subjects were matched both in age ( $p = .139$ ) and gender ( $p = .295$ ). In migraine group 28% MA and 72% MO were included. Two SNPs (rs2052129 and rs10156191) present in *DAO* gene were selected in our study to find any association of theirs with risk for migraine. All the genotypic frequencies both in patients ( $p = .646$ ) and controls ( $p = .295$ ) were in HWE for rs10156191. But for rs2052129 the genotypic frequencies of controls ( $p = .131$ ) were in HWE but for patients ( $p = .041$ ) these were out of HWE. The genotypic and allelic frequency distributions for selected SNPs in this study were given in [Tables 1-2](#).

#### 3.1. Association study of rs2052129 polymorphism

The frequencies of genotypes of SNP rs2052129 were significantly different between patients and controls ( $p = .019$ ). The frequency of TT genotype was 11.2% in migraine patients as compared with 3.2% in controls shown in [Table 1](#). Similarly the frequency of risk allele T was 29.2% in patients which was significant on comparing with that of controls ( $p = .009$ , OR = 1.462; 95% CI: 1.098–1.947). On sub group

**Table 2**  
Genotypic and allelic distribution of rs10156191 polymorphism in this study.

	Genotypic distribution N (%)			Allelic distribution N (%)	
	CC	CT	TT	C	T
Migraine(250)	137(54.8)	98(39.2)	15(6)	372(74.4)	128(25.6)
HC(250)	165(66)	73(29.2)	12(4.8)	403(80.6)	97(19.4)
MO(180)	108(60)	63(35)	9(5)	279(77.5)	81(22.5)
MA(70)	35(50)	30(42.9)	5(7.1%)	100(71.4)	40(28.6)
Male migraine (55)	37 (67.3)	12(21.8)	6(10.9)	86(78.2)	24(21.8)
Female migraine (195)	95(48.7)	88(45.1)	12(6.2)	278(71.3)	112(28.7)
Male control (65)	41(63.1)	19(29.2)	5(7.7)	101(77.7)	29(22.3)
Female control (185)	117(63.2)	56(30.3)	12(6.5)	290(78.4)	80(21.6)

analysis there was variation in frequencies of T allele in MA (28.6%) and MO (27.2%) on comparison with controls (22%) but this difference did not confer any statistical significance as clear in [Table 1](#). In gender analysis frequencies of GT and GG genotypes in male migraine were 16.4 and 76.4% on comparison with that of 40 and 50.8% in control migraine ( $p = .004$ ). In addition, allelic distribution showed significant difference in male migraine samples ( $p = .013$ , OR = 0.443; 95% CI: 0.233–0.840) on comparison with that of male controls. But as the OR < 1, T allele showed a protective effect in male migraine samples. However no such results were reported at allelic level in female migraine patients as shown in [Table 3](#).

#### 3.2. Association study of rs10156191 polymorphism

The distributions of genotypes of SNP rs10156191 differed statistically between controls and migraine ( $p = .002$ ) groups. The frequency of C allele was 74.4% (372C alleles) and for T allele was 25.6% (128 T alleles) in migraineurs as in [Table 2](#). Hence we found significant associations in migraine samples for this SNP at allelic ( $p = .019$ , OR = 1.430; 95% CI: 1.060–1.928) and dominant levels ( $p = .010$ , OR = 1.601; 95% CI: 1.116–1.928) on comparison with controls. On clinical sub grouping of patients, similar trend was observed for MA at genotypic ( $p = .015$ , OR = 1.941; 95% CI: 1.135–3.320) and allelic levels ( $p = .020$ , OR = 1.662; 95% CI: 1.083–2.551). But no such association was reported in MO subgroup. The frequencies of C and T alleles for female migraine were 71.3 and 28.7% which were significant on comparison with 80.6 and 19.4% of female controls. We found similar association in female migraine patients on comparison with female controls both at dominant ( $p = .005$ , OR = 1.811; 95% CI: 1.201–2.729) as well as at allelic levels ( $p = .025$ , OR = 1.460; 95% CI: 1.049–2.033) in gender analysis as shown in [Table 4](#).

### 4. Discussion

We have done a case-control association study in North Indian population for Diamine oxidase polymorphism. This is the first reported case-control study for two SNPs (rs2052129 and rs10156191) in this gene from this population. As women are more prone to develop migraine than men, our study comprised of > 70% females. It has been reported that histamine plays a crucial role in migraine pathophysiology ([Ku et al., 2006](#); [Gazerani et al., 2003](#)) and levels of histamine are higher in migraine patients ([Haimart et al., 1987](#)). There have been several reports of spontaneous release of histamine from leukocytes in migraine patients which are further proved by experimental models that dural mast cells could play a significant role in migraine pathophysiology ([Selmaj, 1984](#)). Much work has not been done in *DAO* SNPs polymorphisms in migraine but studies having other disorders related with histamine such as hyper sensitivity ([Agúndez et al., 2012](#)), rhinitis ([García-Martín et al., 2007](#)) or ulcerative colitis ([García-Martín et al., 2006](#)) have been reported.

In this study, we enrolled 250 migraine patients and 250 healthy controls and reported a significant association of risk of migraine development with both SNPs and with gender. As rs2052129T allele is mainly for decrease activity of *DAO* enzyme expression and could be a cause for high levels of histamine in migraine patients. In concordance with this hypothesis, we reported in the present study that for SNP rs2052129, the frequency of TT genotype was more in patients than that of controls, and rs2052129T allele reported a increased risk for migraine patients as OR = 1.462 with 95%CI = 1.098–1.947. Whereas in subgroup analysis, males migraine with rs2052129T allele showed a protective effect of migraine. Reason behind this may be the selection bias for this allele in male subgroup as patients were not in Hardy-Weinberg equilibrium for this SNP. For rs10156191 a statistically significant difference was reported for T allele at genotypic and allelic levels in migraine patients with that of healthy controls and showed the odds ratio for T allele as 1.430 (95% CI = 1.060–1.928). Similar trend

**Table 3**  
Association study of rs2052129 polymorphism in the studied subjects.

	Genotypic model				Dominant model		Allelic model	
	GT vs GG		TT vs GG		GT + TT vs GG		T vs G	
	p value	OR (95% CI)	p value	OR (95%CI)	p value	OR (95%CI)	p value	OR (95%CI)
Migraine vs HC	0.709	1.074(0.740–1.558)	0.001**	3.924(1.728–8.910)	0.150	1.297(0.910–1.848)	0.009**	1.462(1.098–1.947)
MO vs HC	0.501	1.148(0.768–1.716)	0.032*	2.698(1.091–6.675)	0.226	1.270(0.863–1.869)	0.078	1.326(0.969–1.816)
MA vs HC	0.985	(0.994(0.561–1.763)	0.011*	3.895(1.373–11.050)	0.462	1.222(0.717–2.083)	0.156	1.418(0.929–2.166)
MM vs MHC	0.004**	0.272(0.112–0.659)	0.346	0.524(0.137–2.010)	0.495	0.825(0.475–1.434)	0.013*	0.443(0.233–0.840)
FM vs FHC	0.579	1.126(0.742–1.708)	0.030*	0.314(0.110–0.897)	0.003**	0.349(0.176–0.692)	0.354	0.858(0.620–1.186)

\*p < .05; \*\*p < .01, statistically significant; CI = confidence Interval; OR = odds ratio; MA = migraine with aura; MO = migraine without aura; HC = healthy controls; MM = male migraine; MHC = male healthy controls; FM = female migraine; FHC = female healthy controls.

was reported for T allele for MA (OR = 1.206) and female migraine patients (OR = 1.460). It has been reported in previous studies that women show higher DAO enzyme activity and have higher inter-individual variability than men (Garcia-Martin et al., 2007). Similarly our results revealed association of these SNPs with migraine in gender analysis. These results were also similar to Maintz et al. (2011) who reported the risk for a lower DAO activity with increased frequencies of minor alleles in rs2052129, rs10156191, rs2268999 and rs1049742 SNPs. They found that reporter gene assays at rs2052129 showed a lower promoter activity (p = .016) of the minor allele. The expression of mRNA in DAO was lower (p = .002) in peripheral blood mononuclear cells of homozygous carriers of the minor allele at rs2052129, rs2268999, rs10156191 than that of homozygous carriers of the major allele. Our findings were also similar to that of Garcia-Martin et al. (2015) which reported that defect allele positivity for DAO SNP rs10156191 is 1.61(95% CI = 1.31–2.37) for overall migraine patients and 2.08 for migraine women. Although they showed an increase in frequency of rs2052129G allele which was contradictory with our results as we found higher frequency of rs2052129T allele in migraineurs which is responsible for decrease in enzyme expression in them. Another study by Meza-Velázquez et al. (2017) showed significant association of mutant C2029G DAO SNP polymorphism with migraine women (OR = 1.6; 95% CI = 1.1–2.1). But this nonsynonymous SNP was different from our study. Both the SNPs taken in present study are reported to alter DAO enzyme activity in vivo (Ayuso et al., 2007). Rs10156191T allele encodes a protein with amino acid substitution in the position 16 as Met instead of Thr in wild type protein and reduces intrinsic activity of enzyme and its ability to metabolize circulating histamine (Maintz et al., 2011). As frequency of T allele is more in female migraineurs, it can be expected that decreased clearance of circulating histamine could be the reason for developing migraine. Major drawback of our study was the small sample number. To validate these findings more replication studies on this gene polymorphisms from various ethnical backgrounds with larger sample size is a must.

**Table 4**  
Association study of rs10156191 polymorphism in the studied subjects.

	Genotypic model				Dominant model		Allelic model	
	CT vs CC		TT vs CC		CT + TT vs CC		T vs C	
	p value	OR (95% CI)	p value	OR (95%CI)	p value	OR (95%CI)	p value	OR (95%CI)
Migraine vs HC	0.013*	1.617(1.108–2.360)	0.311	1.505(0.682–3.324)	0.010*	1.601(1.116–2.298)	0.019*	1.430(1.060–1.928)
MO vs HC	0.192	1.318(0.870–1.997)	0.766	1.146(0.467–2.812)	0.203	1.294(0.870–1.924)	0.269	1.206(0.865–1.681)
MA vs HC	0.021*	1.937(1.107–3.392)	0.231	1.964(0.650–5.932)	0.015*	1.941(1.135–3.320)	0.020*	1.662(1.083–2.551)
MM vs MHC	0.410	0.700(0.300–1.635)	0.659	1.330(0.374–4.722)	0.631	0.831(0.390–1.769)	0.927	0.972(0.527–1.793)
FM vs FHC	0.003**	1.935(1.258–2.977)	0.629	1.232(0.529–2.866)	0.005**	1.811(1.201–2.729)	0.025*	1.460(1.049–2.033)

\*p < .05; \*\*p < .01, statistically significant; CI = confidence Interval; OR = odds ratio; MA = migraine with aura; MO = migraine without aura; HC = healthy controls; MM = male migraine; MHC = male healthy controls; FM = female migraine; FHC = female healthy controls.

## 5. Conclusion

This study is the first to report any genetic association of rs2052129 and rs10156191 SNPs in DAO gene in North Indian population. For rs10156191 we reported a significant association at all levels for migraine patients, MA and female subgroup. Hence female patient with rs10156191T allele and in MA subgroup showed an increased risk for migraine. The increase of variants rs2052129T and rs10156191T in patients may be related with reduced DAO gene activity which further is implicated in pathophysiology of migraine. Further studies from various populations and other SNPs in this gene along with a check on DAO serum activity in migraine patients and controls are required to strengthen our results.

## Ethics approval and consent to participate

This study was approved by Ethics Committees from JMI, New Delhi and from that of ESIC Medical College & Hospital, Faridabad. A written informed consent from all the subjects was taken prior to this study.

## Consent for publication

A written consent form from all the subjects was taken that this work is for publication in future.

## Availability of data and materials

Not applicable.

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## Authors' contributions

All authors contributed equally in the preparation of the manuscript and read and approved the final version.

Additional file 1 Figures showing PCR products and restriction digestion products of selected SNPs

## Declaration of Competing Interest

The authors declare that they have no competing interests.

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**Migraines appear more likely to be caused by histamine rather than ethanol.** Schnedl, W. J., & Queissner, R. 2019. European journal of neurology.

DR. WOLFGANG J SCHNEDL (Orcid ID : 0000-0002-5212-5230)

Article type : Letters to the Editor

## **Migraines appear more likely to be caused by histamine rather than ethanol**

Wolfgang J. Schnedl<sup>a</sup> and Robert Queissner<sup>b</sup>

*<sup>a</sup>General Internal Medicine Practice,*

*Dr. Theodor Körnerstrasse 19b, 8600 Bruck, Austria*

*<sup>b</sup>Department of Psychiatry and Psychotherapeutic Medicine,*

*Medical University of Graz, Auenbruggerplatz 36, 8036 Graz, Austria*

### **Correspondence:**

Dr. Wolfgang J. Schnedl  
Professor of Internal Medicine  
General Internal Medicine Practice  
Theodor Körnerstrasse 19b  
A-8600 Bruck/Mur  
Austria  
Phone: +43-3612-55833  
Fax: +43-3612-55833-22  
E-mail: w.schnedl@dr-schnedl.at

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We read with interest the manuscript by Onderwater *et al.* that reported on alcoholic beverages as a trigger factor for migraines [1]. Although, epidemiological studies have found a correlation between alcohol intake and headaches, a specific pathophysiologic mechanism of this headache remains unidentified [2]. Particularly red wine was documented as the most common trigger for migraines in this evaluated population [1]. Most commonly found biogenic amines in wine - histamine, tyramine, phenylethylamine, putrescine, cadaverine, spermidine, serotonin, tryptamine, agmatine - and, flavonoids have suspected relevance for migraines and this implies that ethanol seems not to be the main culprit for the headaches [2].

An unbalanced and elevated quantity of histamine in histamine intolerance (HIT) is assumed to be the consequence of ingesting histamine-containing food or drinks, and mainly the enzyme diamine oxidase's (DAO) reduced ability to metabolize histamine. Generally, red wines reportedly contain clearly more than double the concentrations of biogenic amines, including histamine with  $>2.200\mu\text{g/L}$ , than white wines ( $\sim 900\mu\text{g/L}$  histamine) [3]. Vodka is a clear, distilled alcoholic beverage, made by distilling fermented potatoes or grains that originally have low histamine content [4]. The consumption of vodka was also shown to induce fewer migraines than red wine [1].

DAO genotypes and allelic variants have already shown association with the increased risk for migraines [5]. Recently, a randomized double-blind trial demonstrated that oral ingestion of capsules with DAO reduces headaches in migraine patients [6]. Then, headaches in HIT patients - as one of the many symptoms in HIT - also were, in combination with all other HIT-related symptoms, significantly reduced due to oral supplementation of DAO [7].

In conclusion, the culprit triggers of migraines via consumption of alcoholic beverages are still unknown. However, the histamine content of alcoholic beverages, especially red wine, and possibly parallel consumed food, e.g. mature cheese [4], may play a key role in triggering migraines and headaches.

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Wolfgang J. Schnedl received speaking honoraria from Sciotec. Robert Queissner declares no competing interests.

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**Diamine oxidase (DAO) supplement reduces headache in episodic migraine patients with DAO deficiency: A randomized double-blind trial.** Joan Izquierdo-Casas; Oriol Comas-Basté; M. Luz Latorre-Moratalla; Marian Lorente-Gascón; Adriana Duelo; M. Carmen Vidal-Carou; Luis Soler-Singla **2018** Clinical Nutrition



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## Randomized Control Trials

Diamine oxidase (DAO) supplement reduces headache in episodic migraine patients with DAO deficiency: A randomized double-blind trial<sup>☆</sup>

Joan Izquierdo-Casas<sup>a, b</sup>, Oriol Comas-Basté<sup>c</sup>, M. Luz Latorre-Moratalla<sup>c</sup>,  
 Marian Lorente-Gascón<sup>b</sup>, Adriana Duelo<sup>d</sup>, Luis Soler-Singla<sup>a, b</sup>,  
 M. Carmen Vidal-Carou<sup>c, \*</sup>

<sup>a</sup> Department of Neurology, Hospital General de Catalunya, C/ Pere i Pons 1, 08915, Sant Cugat del Vallès, Spain

<sup>b</sup> Department of Basic Sciences, Universitat Internacional de Catalunya, C/ Pere i Pons 1, 08915, Sant Cugat del Vallès, Spain

<sup>c</sup> Department of Nutrition, Food Sciences and Gastronomy, XaRTA, INSA, School of Pharmacy and Food Sciences, University of Barcelona, Avinguda Prat de la Riba 171, 08921, Santa Coloma de Gramenet, Spain

<sup>d</sup> Department of Nutrition, Instituto Clínico del Déficit de DAO (ICDDAO), C/ Pere i Pons 1, 08195, Sant Cugat del Vallès, Spain

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## SUMMARY

**Background & aims:** Histamine intolerance is a disorder in the homeostasis of histamine due to a reduced intestinal degradation of this amine, mainly caused by a deficiency in the enzyme diamine oxidase (DAO). Among histamine related symptoms, headache is one of the most recorded. Current clinical strategies for the treatment of the symptomatology related to this disorder are based on the exclusion of foods with histamine or other bioactive amines and/or exogenous DAO supplementation. The aim of this study was to assess the efficacy of a food supplement consisting of DAO enzyme as a preventive treatment of migraine in patients with DAO deficiency through a randomized double-blind trial.

**Methods:** 100 patients with confirmed episodic migraine according to current International Headache Society (IHS) criteria and DAO deficiency (levels below 80 HDU/ml) were randomized in two groups. One group received DAO enzyme supplementation and the other received placebo for one month. Clinical outcomes assessed were duration and number of attacks, perception of pain intensity and adverse effects during treatment. The use of triptans was also recorded.

**Results:** Great variability was found in the duration of migraine attacks reported by placebo and DAO groups. A significant reduction ( $p = 0.0217$ ) in hours of pain was achieved in patients treated with DAO supplement, with mean durations of 6.14 ( $\pm 3.06$ ) and 4.76 ( $\pm 2.68$ ) hours before and after treatment, respectively. A smaller reduction without statistical significance was also observed for this outcome in the placebo group, from 7.53 ( $\pm 4.24$ ) to 6.68 ( $\pm 4.42$ ) hours. Only in DAO group, a decrease in the percentage of patients taking triptans was observed. The number of attacks and the scores of pain intensity showed a similar reduction in both groups. No adverse effects were registered in patients treated with DAO enzyme.

**Conclusions:** Migrainous patients supplemented with DAO enzyme during one month significantly reduced the duration of their migraine attacks by 1.4 h. No statistically significant reduction was found in placebo group before and after treatment. The reduction of pain hours observed in placebo group (0.9 h) could explain the lack of significant differences between both study groups. One month of DAO supplementation has demonstrated a positive trend in the improvement of migraine but more studies with a longer treatment period are needed to better assess the efficacy of DAO supplementation.

**Clinical trial registration number:** ISRCTN10091019; [www.isrctn.org](http://www.isrctn.org).

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**Abbreviations:** DAO, Diamine oxidase; IHS, International Headache Society; IQR, interquartile range; HDU, Histamine Degrading Units; NPRS, Numeric Pain Rating Scale.

<sup>☆</sup> This study is listed on the ISRCTN registry with trial ID ISRCTN10091019.

\* Corresponding author.

E-mail address: [mcvidal@ub.edu](mailto:mcvidal@ub.edu) (M.C. Vidal-Carou).

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## 1. Introduction

Histamine is a bioactive amine with essential physiological activities, which can also be found in some common foods in a wide range of concentrations [1,2]. Histamine from diet is principally metabolized in the digestive tract by diamine oxidase (DAO), regulating its presence in the systemic circulation [3]. DAO deficiency could be one of the main causes of histamine intolerance, an alteration in the homeostasis of histamine, which results in a reduced intestinal degradation, and its subsequent increase in plasma [3,4]. DAO deficiency may be congenital; resulting from genetic mutations in DAO gene (chromosome 7q36) that code for an altered protein with low enzymatic activity [5–7], or acquired by certain pathologies that limit DAO secretion, especially in inflammatory or degenerative intestinal disorders [8–10], or by enzymatic blockade by some commonly used drugs [4,5]. Multifaceted clinical symptoms associated with histamine intolerance include headaches, skin reactions such as urticaria and pruritus, gastrointestinal disorders as flatulence, diarrhea, nausea and abdominal pain, sneezing, rhinorrhea, arrhythmias, hypotension and muscle aches [4,11]. Headache is one of the symptoms most frequently related to histamine intolerance. Reduced DAO activity has been described by several clinical studies in patients diagnosed with some pathology such as atopic eczema, chronic urticaria, chronic abdominal pain or inflammatory bowel diseases [9,10,12–15]. A recent study also reported a high prevalence (87%) of DAO deficiency in a group of 137 patients diagnosed with migraine [16].

Migraine is a neurological and disabling pathology with a multifactorial etiology that can negatively impact both family and work activities. Its prevalence in Spanish population has been estimated to be 12% [17]. Physiopathological mechanisms supporting the onset of migraine are complex, but several pathways have been described to explain the association between histamine and headache. In the nervous system, certain neurons synthesize histamine in the posterior-basal hypothalamic nuclei, an area recently postulated as the locus of diverse primary headaches due to increased activity detected during the prodromal phases of migraine attacks [18]. Although it was first thought that histamine did not cross the blood–brain barrier, it seems that it may stimulate hypothalamic activity through the circumventricular organs, which lack this barrier [18]. For this reason, high plasmatic histamine levels could originate an increase of histamine in hypothalamus. Moreover, neurogenic inflammation involves the release of histamine, which, in turn, promotes the release of substance P and the gene-related peptide, both closely linked to the pain process in migraine patients [19]. On the other hand, histamine could also induce a vascular headache, since its plasmatic increase would provoke a release of nitric oxide upon stimulation of H1R receptors found in intracranial arteries [4].

DAO deficiency is responsible of plasmatic histamine accumulation, thus hypothetically could be one of the migraine triggers. The aim of this study is to assess the efficacy of DAO supplementation as a preventive treatment in migraine patients with DAO deficiency through a randomized double-blind trial.

## 2. Methods

### 2.1. Subjects

A double-blind randomized study was carried out with 100 patients with confirmed episodic migraine diagnosis according to current International Headache Society (IHS) criteria [20] and DAO deficiency.

A total of 139 participants diagnosed with migraine were recruited by the Headache Unit of the Hospital General de

Catalunya (Sant Cugat del Vallès, Barcelona). After DAO activity determination, 119 of them were eligible candidates to be included in the study (migraine diagnosis and DAO deficiency). Finally, 100 patients were selected by applying additional criteria. Inclusion criteria were the age between 18 and 65 years old and 4 to 14 migraine episodes/month for a minimum of six months prior to study start. Exclusion criteria were: the onset of migraine over 50 years old, the diagnosis of other kind of headache in the same patient, pregnancy and the following of a preventive treatment for episodic migraine during three months prior to the study.

DAO activity was determined from plasma samples with an enzymatic immunoassay method (D-HIT, Sciotec, Austria) after an 8-h fasting period. Serum DAO levels below the cut-off value of 80 HDU/ml were considered as DAO deficient.

### 2.2. Outcome measures

Clinical outcomes assessed were duration and number of migraine attacks, perception of pain intensity and adverse effects during treatment. The duration of migraine attacks was measured by the number of hours of pain. Pain intensity was assessed by the Numeric Pain Rating Scale (NPRS), a 10-point grading scale where 0 represents absence of pain and 10 is the worst possible pain.

Other complementary information has been also recorded, such as adverse effects during treatment and the use of specific analgesic drugs, specifically the intake of triptans during migraine attacks. Data about the number of triptans in each migraine attack was recorded per patient, classifying their intake as low (less than 5 capsules/month), moderate (between 6 and 10 capsules/month) and high (more than 10 capsules/month).

### 2.3. Study protocol

Figure 1 provides details of the study design. In baseline consultation (C0) patients received a first diary to record data related to the outcome measures during one month prior to the study. After one month, all patients were scheduled for the first consultation (C1) and submitted the completed first diary. Patients were then randomized in two groups (DAO and placebo) and received a second diary to record the information requested during the month of treatment, which they submitted in a second consultation (C2).

Randomization in two groups was double-blind using the RANUNI procedure (SAS, v. 6.12, SAS Institute, Cary, NC, USA). One group received DAO enzyme supplementation ( $n = 50$ ) and the other placebo ( $n = 50$ ). Stratification considering age and sex was applied to assure similarity in baseline characteristics of both groups. The treatment consisted in the oral administration of 2 capsules of DAO supplement or placebo 20 min before breakfast, lunch and dinner. Each capsule contained 4.2 mg of porcine kidney protein extract with 7% of DAO with an enzymatic activity of 10,000 HDU/ml. The content of the capsules was microencapsulated with a gastroresistant shellac coating. Placebo consisted of microcrystalline cellulose and gelatin capsules with the same form, size and color than DAO ones and was provided by the same manufacturer. Treatment compliance was verified by counting the remaining capsules for each patient.

Table 1 shows the characteristics (sex, age and DAO activity) of all patients included in both study groups.

The Ethics Committee of the Hospital General de Catalunya approved the study and all participants signed an informed consent form.

### 2.4. Statistical analysis

The size of the sample was calculated assuming a 5% type 1 error rate and a desired statistical power of 80%. Taking into account a

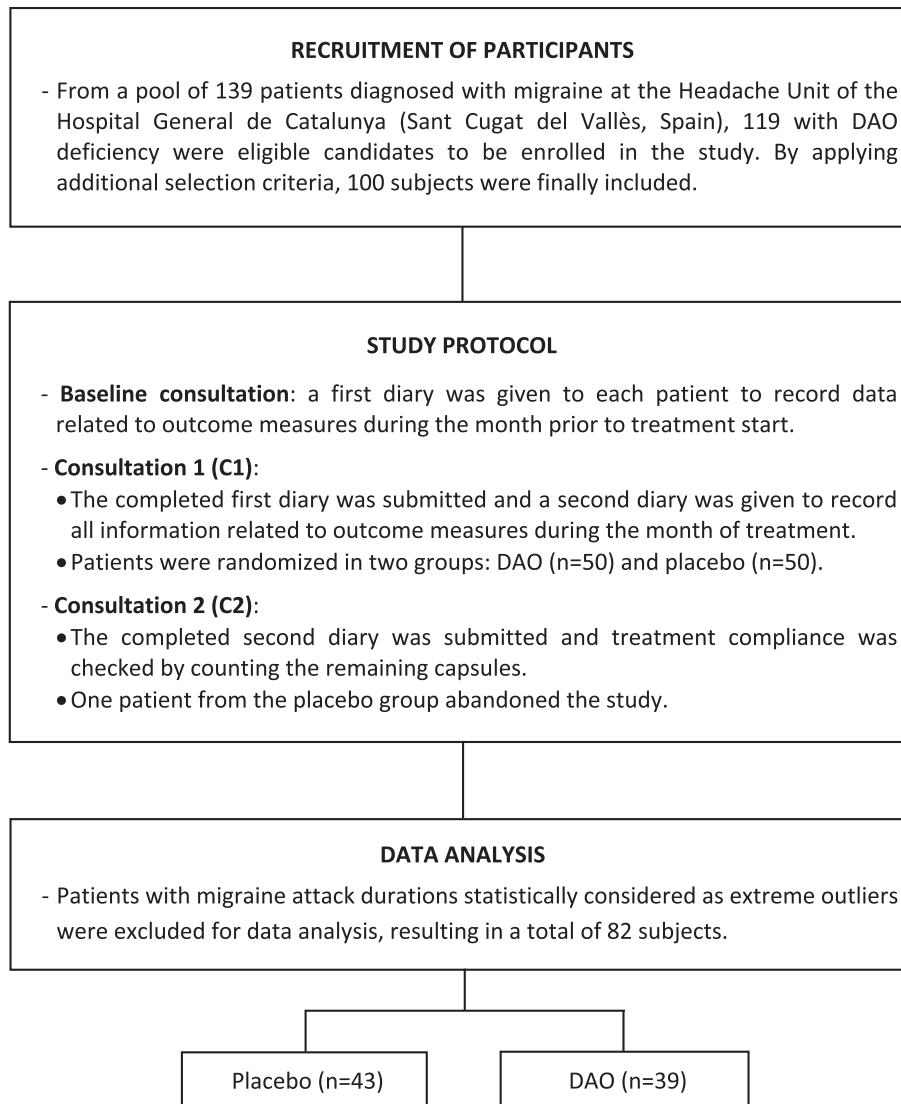


Fig. 1. Schematic representation of the study design.

**Table 1**

Characteristics (sex, age and DAO activity levels) of all patients included in both placebo and DAO groups.

Variable		Placebo N = 43	DAO N = 39	Total N = 82
Sex (number of patients)	Female	36	32	68
	Male	7	7	14
Age (years old)	Mean (SD)	43.6 (11.0)	40.8 (10.9)	42.3 (11.0)
	min–max	23.8–64.9	18.4–61.2	18.4–64.9
DAO activity (HDU/ml)	Mean (SD)	54.8 (11.3)	53.3 (11.2)	54.1 (12.9)
	min–max	18.6–79.6	22.6–79.0	18.6–79.6

SD: standard deviation; min: minimum; max: maximum; HDU: histamine degrading units.

minimal detectable mean difference of 1.25 attacks/month with a SD of 2, a total of 82 subjects would be needed. Based on an expected 15% dropout rate, a total of 100 patients (50 per group) were included to assure that at least 82 subjects would complete the full study.

T-test was used to evaluate differences within groups before (C1) and after (C2) treatment and between groups (placebo and DAO) in migraine attack duration (hours), number of attacks, and

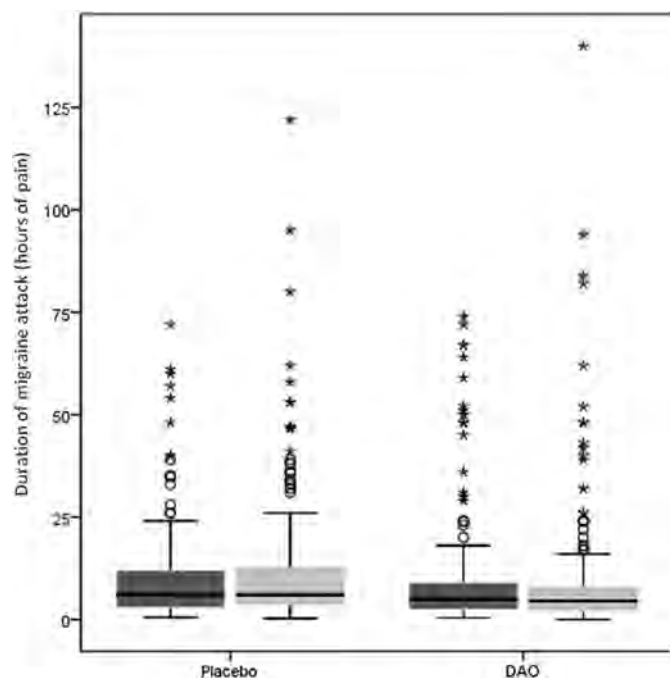
pain scales. Chi-squared test was used to determine significant differences in patients taking triptans. Probability values of  $p < 0.05$  were accepted as significant. Statistical analysis was performed using a SPSS for Windows, version 22 (Chicago, IL).

### 3. Results

No significant differences in treatment compliance were observed between both groups ( $p = 0.543$ ), with a mean (SD) of remaining capsules of 17 (20.6) and 19.9 (24.7) in placebo and DAO group, respectively.

Great variability was found in the duration of migraine attacks reported by placebo and DAO groups, with some extremely high values recorded (Fig. 2). Values exceeding that obtained by the formula  $P_{75} + 3 \cdot IQR$  ( $IQR$ : interquartile range;  $P_{75} - P_{25}$ ) were statistically considered as extreme outlier values, and the corresponding patients were excluded, resulting in a final sample of 82 subjects, 43 in the placebo and 39 in the DAO group.

Table 2 shows the duration of migraine attacks, as the mean of pain hours for each patient (total number of pain hours/number of attacks) before and after treatment. Before treatment, mean (SD)



**Fig. 2.** Box plot for the duration of migraine attacks (in hours of pain) in placebo and DAO groups one month before (C1) and after one month of treatment (C2). The bottom and top of the box (interquartile range) are the percentile 25 and the percentile 75, respectively. Central line represents the median. Lines extending vertically from the boxes (whiskers) indicate variability outside the interquartile range. Outliers are plotted as circles and extreme outliers as asterisks.

values were 7.5 h (4.2) and 6.1 h (3.1) for placebo and DAO group, respectively. A statistically significant reduction in the duration of pain was obtained in DAO group after one month of treatment ( $p = 0.0217$ ). Although to a smaller extent, a reduction in this outcome was also observed in placebo group, but without statistical significance. Regarding the difference between groups, patients treated with DAO supplement reached a higher reduction of

migraine attack duration (1.4 h) than in placebo group (0.9 h), although with a lack of statistical significance. When considering the number of attacks, significant reduction was found both in DAO group (2.67 attacks,  $p = 0.0004$ ) and in placebo group (2.16 attacks,  $p = 0.0059$ ). Similar reductions in the frequency of migraine attacks were achieved in both study groups. Regarding pain intensity, similar scores were found before and after the treatment in both groups (Table 2).

No adverse effects were registered in patients with DAO supplementation. Only one patient of the placebo group described gastrointestinal troubles and subsequently abandoned the study.

Figure 3 shows the percentage of patients who used triptans before and after the treatment with placebo or DAO. A reduction of patients taking triptans was observed in DAO group whereas in placebo group there was an increase in patients with triptans intake. Figure 4 illustrates the intake of triptans before and after one month of treatment with placebo or DAO for each of the patients that reported consumption. In fact, among individuals treated with DAO, 44% of them reduced triptans intake (5 reported a total withdrawal), 30% remained unchanged and 26% increased the intake of these drugs. On the contrary, an increase in triptans intake in the placebo group was recorded in 57% of the individuals. However, in neither case the association was statistically significant.

#### 4. Discussion

To our knowledge, this is the first double-blind randomized trial to assess the effect of DAO supplementation in migraine patients. Currently the main measure to prevent or mitigate the symptomatology related to histamine intolerance by DAO deficiency is the dietary management avoiding foods with high contents of histamine or other bioactive amines also metabolized by DAO, such as putrescine and cadaverine, as well as foods described as histamine-releasing [4,5,21]. Several intervention studies have demonstrated the effectiveness of histamine restrictive diets, some of them specifically focused on headache, with positive results in most of the patients involved in each study [12,13,22,23]. However, the wide and variable distribution of histamine in foods together with other

**Table 2**

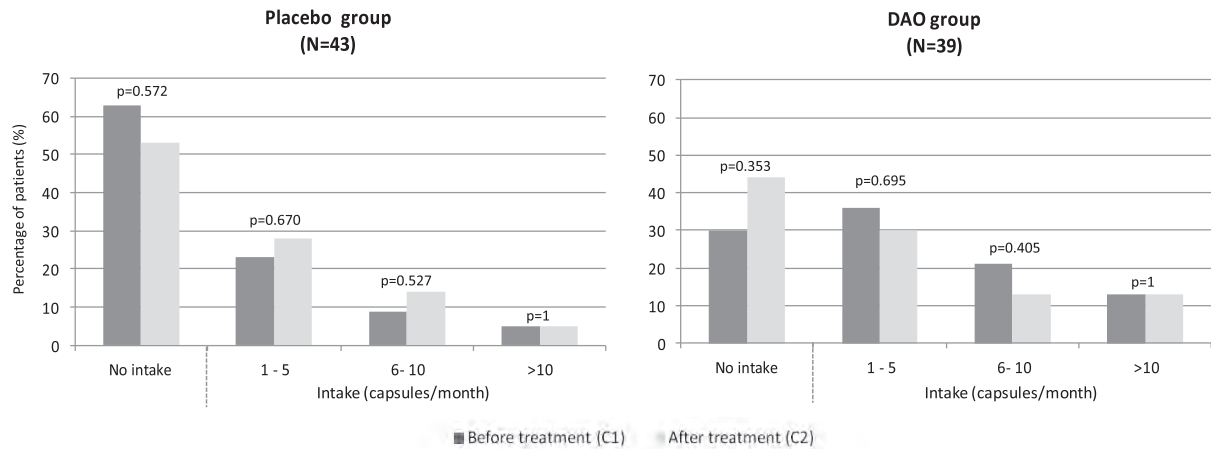
Duration, number and pain intensity of migraine attacks in placebo and DAO groups before (C1) and after one month of treatment (C2).

			Placebo N = 43	DAO N = 39	$p^a$
Duration of attacks (hours)	Before treatment (C1)	Mean (SD)	7.53 (4.24)	6.14 (3.06)	0.6040
		min–max	0 to 21	1.8 to 15	
	After treatment (C2)	Mean (SD)	6.68 (4.42)	4.76 (2.68)	
		min–max	0 to 16.5	0 to 11	
C2–C1		Mean (SD)	–0.85 (5.35)	–1.38 (3.60)	
		min–max	–15.2 to 10.5	–15.0 to 6.6	
Number of attacks/month	Before treatment (C1)	Mean (SD)	9.26 (5.21)	10.18 (4.44)	0.6172
		min–max	0 to 20	1 to 20	
	After treatment (C2)	Mean (SD)	7.09 (4.97)	7.51 (4.97)	
		min–max	0 to 20	0 to 19	
C2–C1		Mean (SD)	–2.16 (4.88)	–2.67 (4.26)	
		min–max	–15.0 to 8.0	–15.0 to 5.0	
Pain intensity (NPRS score)	Before treatment (C1)	Mean (SD)	5.62 (1.57)	5.27 (1.43)	0.3424
		min–max	0 to 9	1.4 to 7.9	
	After treatment (C2)	Mean (SD)	4.88 (2.31)	5.07 (2.02)	
		min–max	0 to 8.5	0 to 8.6	
C2–C1		Mean (SD)	–0.71 (2.67)	–0.21 (1.98)	
		min–max	–7.8 to 7.8	–7.0 to 2.0	

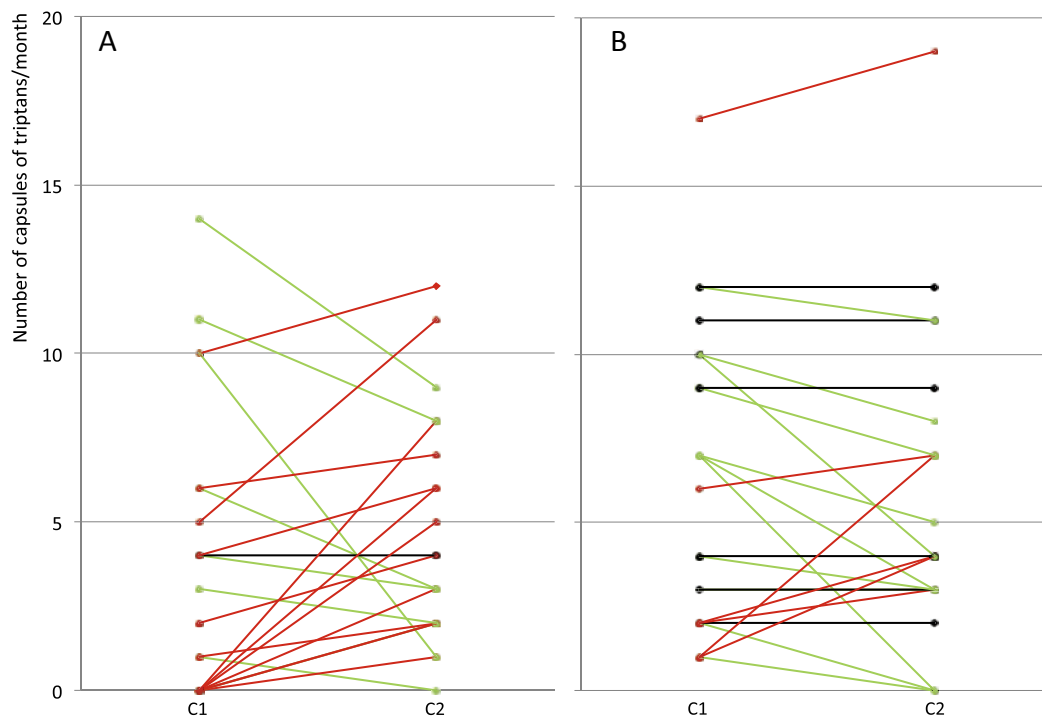
SD: standard deviation; min: minimum; max: maximum; NPRS: Numeric Pain Rating Scale.

<sup>a</sup>  $p$ -value for the statistical differences between placebo and DAO groups.

<sup>b</sup>  $p$ -value for the statistical differences between before (C1) and after (C2) treatment.



**Fig. 3.** Percentage of patients who used triptans before (C1) and after one month of treatment (C2) with placebo or DAO. The triptans intake classification was: no intake, low (1–5 capsules/month), moderate (between 6 and 10 capsules/month) and high (more than 10 capsules/month).



**Fig. 4.** Number of capsules of triptans required before (C1) and after (C2) one month of treatment with placebo (A) or DAO supplementation (B) for each of the patients that reported consumption. Red line means an increase, green line means a decrease and black line means no change in the triptan intake after one month of treatment.

additional restrictions (histamine-releasing foods and presence of other bioactive amines) makes difficult the diet adherence and increases the risk of nutritional imbalances. The use of DAO supplementation could be an alternative to a histamine-free diet or be useful to make easier its compliance.

In our double-blind randomized clinical study, DAO enzyme supplementation consisted in porcine kidney extract micro-encapsulated with a shellac gastroresistant coating that guaranteed its resistance in the pH of the stomach and subsequently its hydrolysis by pepsin. Intestinal conditions allow the release of the unaltered enzyme that will potentially resist the proteolytic attack of trypsin and chymotrypsin. In fact, Federico et al. [24] reported a

high in vitro stability of DAO of plant origin in the presence of trypsin, with 100% of the enzymatic activity during the first 45 min.

The administration of the DAO supplement was able to significantly reduce the mean duration of migraine attacks by 1.4 h (from an initial mean value of 6.1 h). The duration of migraine attacks has been used as an efficacious measure to assess the clinical and quality of life impact of the treatment [25–27]. On the other hand, the effect of this supplementation in the migraine frequency could not be established because both study groups showed a significant reduction greater than 2 monthly attacks. Moreover, the lack of significance observed between groups could be explained by the limited duration of one month of treatment. A longer intervention

period might confirm the trend observed in the current study about the effects of DAO supplementation on migraine improvement.

Regarding pain intensity, no differences were found based on the NPRS score reported by the subjects from both groups. The usefulness of this scale has been questioned in patients with long-term pain because they tend to score between 4 and 6 (golden section) [28]. Likewise, the use of triptans (allowed in this study), could cover up the real perception of pain.

Patients with DAO deficiency could be stratified as reduced DAO activity level (40–80 HDU/ml) and markedly reduced DAO activity level (<40 HDU/ml). In this study, only few individuals showed a markedly reduced DAO activity, with 7 and 4 patients in the placebo and DAO group, respectively. The reduced number of subjects with markedly reduced DAO activity, which did not allow the stratification of these patients, together with the fact that all subjects received the same dose of treatment, could diminish the strength of the current study. Markedly reduced DAO activity patients could require a higher dose to reach the improvement of symptomatology.

A trend to diminish the triptans intake was observed in DAO group, with some patients that abandoned the use of triptans. A reduction of the number of patients with low and moderate intake of these drugs was also observed. The reduction in triptans intake could indicate a decrease in the intensity of pain during migraine crisis. This trend was not observed in the placebo group where the intake of triptans increased in comparison with the baseline.

There are few studies about the efficacy of DAO supplementation in the improvement of headache and other symptoms associated with histamine intolerance [29,30], and all of them with a lower number of patients involved. A randomized double-blind crossover provocation study [29], using histamine containing and histamine-free tea in combination with DAO capsules or placebo in 39 patients with histamine intolerance revealed that two capsules of DAO supplementation reduced the appearance of headache, skin reactions, and respiratory and gastrointestinal disorders in comparison with placebo. Recently, Manzotti et al. [30] also observed that DAO supplementation for a period of at least two weeks was associated with a reduction of one or more of the reported symptoms in 13 out of 14 patients with histamine intolerance. When DAO supplementation was associated with low-histamine diet a higher reduction of symptoms was reported in comparison to the diet alone. However, in this study no placebo group was considered.

In the current trial the efficacy of DAO supplementation was assessed after only one month of treatment, so probably, a longer treatment period could lead to a great improvement. Moreover, no complementary dietary intervention was considered, and then it is possible that DAO supplementation together with histamine free-diet could enhance the results of the treatment. Likewise, in further studies it would be of interest to segregate patients in different grades of DAO deficiency. Therefore, more randomized placebo-controlled studies, preferably with a cross-over design, are needed to assess the benefits of DAO supplementation for a longer treatment period, and the combined effect of DAO supplement together with histamine free diet, which would make diets less restrictive and improve patients quality of life.

### Sources of support

DR Healthcare (Barcelona, Spain) has provided DAO supplement and placebo capsules. Oriol Comas-Basté is a recipient of a doctoral fellowship from the University of Barcelona (APIF 2015).

### Conflict of interest

None of the authors reported a conflict of interest related to the study.

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2018.01.013>

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**Low-histamine diet supplemented with exogenous diamine oxidase enzyme is useful for treating migraine in patients with DAO Deficiency** A.Duelo; M.Berbel; H.Mantecon-Laviguerie; O.Comas-Basté; M.L.Latorre-Moratalla; M.T.Veciana-Noguès; M.C.Vidal-Carou. **2018** Spanish Nutrition Society SEÑ and the Catalan Association of Food Science (ACCA), Spain

# **Abstracts of the XVII Conference of the SEÑ and the X Meeting of the ACCA**

## **Spanish Nutrition Society (SEÑ) and the Catalan Association of Food Science (ACCA)**

Barcelona, Spain, June, 27–29, 2018

### **Abstracts**

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## Oral Abstract Presentations

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### WHAT ABOUT LEGUMES AS A PLANT SOURCE OF THE DAO ENZYME?

O. Comas-Basté; S. Sánchez-Pérez; R.I. Garza-Guajardo; M.L. Latorre-Moratalla; M.T. Veciana-Nogués; M.C. Vidal-Carou.

Departament de Nutrició, Ciències de l'Alimentació i Gastronomia. Facultat de Farmàcia i Ciències de l'Alimentació. INSA-UB. XaRTA. Universitat de Barcelona. Santa Coloma de Gramenet. Spain.

**Introduction:** The use of exogenous diamine oxidase (DAO) enzyme has been recently postulated as a potential strategy for the treatment of histamine intolerance, a disorder in the homeostasis of histamine caused by a reduction of its intestinal degradation. Dietary supplements based on gastro-resistant encapsulated porcine kidney protein extract are available on the market to reduce the symptoms of this intolerance. Although with scarce scientific research, there are some references about the potential of pea seedlings as a source of DAO.

**Objectives:** To study the capacity of legumes and their sprouts to reduce histamine in vitro and evaluate the influence of different growing conditions on this enzymatic activity. If confirmed, legumes could become an advantageous alternative to porcine DAO enzyme from a productive and sustainable perspective.

**Methods:** In vitro DAO activity was measured through an enzymatic assay and the subsequent analysis of remaining histamine by UHPLC-FL. Analysed samples were both raw pulses and sprouts of lentils, beans (white, red and black), broad beans, peas, chickpeas and soy.

**Results:** Histamine-degrading capacity was found both in raw pulses and sprouts of some legumes. Lentils, broad beans and white, red and black beans showed in vitro DAO activity, ranging from 0.32 to 1.95 mU/g (nmols of degraded histamine per minute/g of legume). This activity was absent in raw peas, chickpeas and soy. Etiolated sprouts (grown in darkness) of various legumes showed higher DAO activity in comparison with raw pulses, with mean values of 40.25±8.1 mU/g in chickpea sprouts, 35.2±5.3 mU/g in lentil sprouts and 27.5±1.3 mU/g in pea sprouts.

**Conclusions:** Results confirm the ability of various raw pulses and legume sprouts to reduce histamine in vitro and their potential as a plant source of the DAO enzyme. Further studies are needed to better establish the influence of growing conditions of the sprouts and extraction treatments on DAO activity.

**Conflict of interest:** Authors declare no conflict of interest.

**Key words:** histamine / histamine intolerance / DAO / legumes / sprouts.

098

### LOW-HISTAMINE DIET SUPPLEMENTED WITH EXOGENOUS DIAMINE OXIDASE ENZYME IS USEFUL FOR TREATING MIGRAINE IN PATIENTS WITH DAO DEFICIENCY

<sup>(1)</sup>A. Duelo; <sup>(1)</sup>M. Berbel; <sup>(1)</sup>H. Mantecon-Laviguerie; <sup>(2)</sup>O. Comas-Basté; <sup>(2)</sup>M.L. Latorre-Moratalla; <sup>(2)</sup>M.T. Veciana-Nogués; <sup>(2)</sup>M.C. Vidal-Carou.

<sup>(1)</sup>Department of Nutrition. Instituto Clínico del Déficit de DAO (ICDDAO). Sant Cugat del Vallès. Spain; <sup>(2)</sup>Departament de Nutrició, Ciències de l'Alimentació i Gastronomia. Facultat de Farmàcia i Ciències de l'Alimentació. INSA-UB. XaRTA. Universitat de Barcelona. Santa Coloma de Gramenet. Spain.

**Introduction:** Low-histamine diets and/or exogenous diamine oxidase (DAO) supplementation are currently used to treat symptoms of histamine intolerance (IH), a disorder in histamine homeostasis that increases its plasma levels, mainly due to DAO deficiency. Headache is the most recognized symptom.

**Objectives:** To assess the effectiveness of a low-histamine diet plus a DAO enzyme supplement on the remission of migraine in subjects with DAO deficiency.

**Methods:** An intervention study was carried out in 212 individuals with a migraine diagnosis by a neurologist according to the International Classification of Headache Disorders and with DAO deficiency (DAO <80 HDU/ml). Subjects followed a 3-month low-histamine diet based on the exclusion of foods considered rich in histamine or other biogenic amines and usually related to the onset of HI symptoms, such as fermented products (cheese,

dry-fermented sausage, wine, beer), semi-preserved and canned fish, shellfish, certain fruits and vegetables (spinach, tomato, eggplant, avocado, citrus, bananas, strawberries, pineapple, nuts) and chocolate<sup>1,2</sup>. A DAO supplement was administered before breakfast, lunch and dinner. Outcomes assessed were duration and number of attacks and perception of pain intensity with a score-scale from 0 (absence) to 10.

**Results:** Most subjects showed an improvement in migraines after the 3-month treatment: 34.9% reported complete remission and another 35.8% had a reduced number of migraine episodes per month, of less duration and pain intensity. The treatment was less successful in 29.3% of patients. On average, when comparing baseline and final values after treatment, all outcomes were significantly reduced: 8 to 2 attacks per month, 24 to 3 hours of pain and 8 to 4 in pain intensity scoring.

**Conclusions:** A low-histamine diet supplemented with the DAO enzyme for three months was useful in reducing the number of attacks, duration and intensity of pain in migraineous patients with DAO deficiency.

**References:** <sup>1</sup>Rosell-Camps et al. (2013); <sup>2</sup>Wagner et al. (2017).

**Conflict of interest:** Authors declare no conflict of interest.

**Key words:** DAO / histamine / low-histamine diet / migraine.

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## Poster Abstract Presentations

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099

### FOOD ALERT NETWORK AND ITS RELATIONSHIP WITH ADVERSE REACTIONS TO FOOD

*V. Torres; J. García; J.A. Sánchez-Rodríguez; L. Macías.*

Unidad de Protección de la Salud. Área Sanitaria Norte de Málaga. Hospital de Antequera. Antequera. Spain.

**Introduction:** In Andalucía, the possibility of suffering an anaphylactic shock due to food intake has quadrupled in the last two decades. Adverse reactions due to allergies and food intolerances are thus a public health problem in our Autonomous Community.

We have distinguished 14 substances with a higher risk of causing food allergy, so their inclusion in nutrition labeling is mandatory. Since 2008, specific allergen control programs have been carried out on foods marketed in Andalucía.

**Objectives:** To analyze the situation of food alerts due to the presence of undeclared allergens as a basis for action in those sectors with the greatest involvement. To contribute to a high degree of health protection against food-borne risks, and to promote the quality of the environment where people live

**Methods:** Source of information: Database of alerts network of the Área Sanitaria Norte de Málaga (ASNM). Categorization of foods according to the Guide for the Operation of the General Sanitary Registry of Food and Food Companies.

Procedure of action according to the ALERT PROCESSING Manual (Ministry of Health of the Junta de Andalucía).

**Results:** The number of alerts in our ASNM related to allergens has risen significantly in recent years. The foods with the greatest implication are of plant origin (cereals).

**Conclusions:** The increase of alerts coincides with the implementation of Regulation 1169/2011 and is related to the agri-food characteristics of our Autonomous Community.

**Conflict of interest:** Authors declare no conflict of interest.

**Key words:** food allergy / public health / alerts.

**Low serum diamine oxidase (DAO) activity levels in patients with migraine.** Izquierdo-Casas J1,2, Comas-Basté O3, Latorre-Moratalla ML3, Lorente-Gascón M2, Duelo A4, Vidal-Carou MC5, Soler-Singla L1,2. **2017**- J Physiol Biochem. doi: 10.1007/s13105-017-0571-3.

# Low serum diamine oxidase (DAO) activity levels in patients with migraine

Joan Izquierdo-Casas<sup>1,2</sup> · Oriol Comas-Basté<sup>3</sup> · M. Luz Latorre-Moratalla<sup>3</sup> · Marian Lorente-Gascón<sup>2</sup> · Adriana Duelo<sup>4</sup> · M. Carmen Vidal-Carou<sup>3</sup> · Luis Soler-Singla<sup>1,2</sup>

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**Abstract** Histamine intolerance is a disorder in the homeostasis of histamine due to a reduced intestinal degradation of this amine, mainly caused by a deficiency in the enzyme diamine oxidase (DAO). Among the several multi-faced symptoms associated with histamine intolerance, headache is one of the most recognized and disabling consequences. The aim of this study was to determine the prevalence of DAO deficiency in patients with a confirmed migraine diagnosis according to the current International Headache Society (IHS) and in non-migraine subjects. DAO activity was assessed in a total of 198 volunteers recruited at the Headache Unit of the Hospital General de Catalunya, 137 in the migraine group and 61 as a control group. DAO enzyme activity in blood samples was determined by ELISA test. Values below 80 HDU/ml (Histamine Degrading Unit/ml) were considered as DAO deficient. Mean value of DAO activity from migraine population ( $64.5 \pm 33.5$  HDU/ml) was significantly lower ( $p < 0.0001$ ) than that obtained from healthy volunteers ( $91.9 \pm 44.3$  HDU/ml). DAO deficiency was more prevalent in migraine patients than in the control group. A high incidence rate of DAO

deficiency (87%) was observed in the group of patients with migraine. On the other hand, 44% of non-migrainous subjects had levels of DAO activity lower than 80 HDU/ml. Despite the multifactorial aetiology of migraine, these results seem to indicate that this enzymatic deficit could be related to the onset of migraine.

**Keywords** Headache · Migraine · Histamine · Diamine oxidase (DAO) · Histamine intolerance

## Introduction

Diamine oxidase (DAO), also called histaminase, is one of the main enzymes in the metabolism of histamine, playing an important role in the degradation of this amine in the intestinal epithelium, regulating its passage into the systemic circulation. A reduced DAO activity could be one of the causes of histamine intolerance, a disorder in the homeostasis of histamine, which provokes the accumulation of this amine in plasma and the appearance of multi-faced allergy-like clinical symptoms. DAO deficiency may be the result of a genetic mutation [1, 7] or related to certain diseases that limit the secretion of this enzyme, especially inflammatory or degenerative intestinal disorders [9, 15]. Finally, certain medications can also cause a specific and reversible inhibition of DAO activity [14, 17].

Unlike the well-known histamine intoxication, appearing after consumption of products with high histamine contents, histamine intolerance symptoms may appear even after the intake of low amounts of this amine [5]. Consequently, the dietary management is the main clinical tool to prevent the symptomatology related to histamine intolerance, based on the follow up of histamine-free diets [3, 24, 29, 30]. Apart from histamine, the presence of other bioactive amines, such

✉ M. Carmen Vidal-Carou  
mcvidal@ub.edu

<sup>1</sup> Department of Neurology, Hospital General de Catalunya, C/ Pere i Pons 1, 08915 Sant Cugat del Vallès, Spain

<sup>2</sup> Department of Basic Sciences, Universitat Internacional de Catalunya, C/ Pere i Pons 1, 08915 Sant Cugat del Vallès, Spain

<sup>3</sup> Department of Nutrition, Food Sciences and Gastronomy, XaRTA, INSA, School of Pharmacy and Food Sciences, University of Barcelona, Avinguda Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain

<sup>4</sup> Department of Nutrition, Instituto Clínico del Déficit de DAO (ICDDAO), C/ Pere i Pons 1, 08195 Sant Cugat del Vallès, Spain

as putrescine, could be co-responsible of the triggering of adverse health effects by competing for the same metabolic pathway [4, 14]. In addition, although there is lack of evidence about the mechanism, certain foods have been associated with an endogenous ability to release histamine, such as egg white, citrus, chocolate and crustaceans [27]. More recently, the supplementation with exogenous DAO enzyme has been postulated as a complementary preventive treatment for histamine intolerance, improving the quality of life of patients undergoing those dietary restrictions [13, 14].

Symptoms associated with the accumulation of histamine in plasma may occur due to the actions of histamine in multiple organs according to the expression of histamine receptors, including gastrointestinal tract, lung, skin, cardiovascular system and brain. Therefore, the main symptoms described for histamine intolerance are headache, flatulence, diarrhoea, abdominal pain, sneezing, rhinorrhea, hypotonia, arrhythmias, idiopathic urticaria and pruritus [14, 17]. Although there is no general consensus on histamine intolerance diagnosis, the most commonly used diagnostic algorithm includes the presentation of at least two of these symptoms and the clinical improvement after following a histamine-free diet. Negative results for food allergen-specific IgE are also required [14, 17].

Headache is one of the most recognized and disabling consequences of histamine intolerance [25, 31]. Migraine is a chronic neurovascular disorder that may be caused by several triggers (physiological, hormonal, behavioural, environmental and nutritional) as has been recently reported by Kokavec [12]. In patients diagnosed with migraine, increased plasmatic levels of histamine were reported during and among attacks [14]. According to Maintz and Novak [17], the association between headache and DAO deficit could be explained because the enzymatic deficiency would provoke an increase of plasmatic histamine that would be responsible for the appearance of headaches by releasing nitric oxide upon stimulation of H1R receptors found in intracranial arteries. In addition, a high DAO production by the placenta could potentially explain the improvement of migraine that some women experience during pregnancy [18].

Clinical studies have shown an association between a reduced DAO activity and some of the above-mentioned symptoms related to histamine intolerance. Mušič et al. [22] reported that 80% of 316 patients with suspected histamine intolerance showed a reduced serum DAO activity. Moreover, mean DAO activity levels of these patients were significantly lower than in healthy controls. Likewise, the study carried out by Manzotti et al. [19] evaluated DAO activity in 14 patients with a potential diagnosis for histamine intolerance, with the most reported symptoms being functional bloating, abdominal pain, tachycardia, diarrhoea, headache, pruritus, flushing, rhinorrhea or nausea. In this case, it was found that 71% of patients had serum DAO activity under the threshold

considered as cut-off for histamine intolerance with a mean DAO activity value significantly lower than healthy controls. Apart from these studies dealing with patients with coexisting histamine intolerance symptoms, other clinical studies have correlated DAO deficiency with some specific pathologies, mainly gastrointestinal and dermatological complaints [6, 8, 9, 16, 21, 23, 25, 26]. However, according to our knowledge, there is little information available about serum DAO levels in patients clinically diagnosed with migraine. The aim of this study was to determine the prevalence of DAO deficiency in patients with a confirmed episodic migraine diagnosis according to the current International Headache Society (IHS) and in non-migraine subjects.

## Material and methods

### Subjects of the study

The study was performed in the Headache Unit of the Hospital General de Catalunya (Sant Cugat del Vallès, Barcelona, Spain) with a total of 198 adult volunteers aged between 18 and 65 years. Episodic migraine, as established by the IHS in the International Classification of Headache Disorders, is mainly characterized by the presence of 0 to 14 headache days per month. Two different groups were considered: a migraine group including 137 patients (122 females [89%] and 15 males [11%]) diagnosed according to current IHS criteria [10] and a control group of 61 volunteers (34 females [56%] and 27 males [44%]) without clinical criteria for migraine. For the migraine group, individuals with the onset of migraine over 50 years old, the diagnosis of other kind of headache, the possibility of pregnancy and the following of a preventive treatment for episodic migraine during 3 months prior to the study were excluded. The mean age of patients with migraine was 41.95 years ( $\pm 11.3$ ) and for control volunteers, it was 42.46 years ( $\pm 14.4$ ) (Table 1).

The Ethics Committee of the Hospital General de Catalunya approved the study, and all participants signed an informed consent form. This study is listed on the ISRCTN registry with trial ID ISRCTN10091019.

**Table 1** Information about study subjects including migraine and control groups

Characteristic	Migraine group	Control group
N	137	61
Age (mean)	41.95	42.46
Gender (%)		
Female	89	56
Male	11	44

**DAO activity analysis**

Blood samples were collected from all subjects by venipuncture in an EDTA tube after an 8-h fasting period, and samples were analysed with ELISA to determine DAO enzyme activity in accordance with the manufacturer instructions (D-HIT, Sciotec, Austria). This method was previously used for the same purpose by Mušič et al. [22]. Values above 80 HDU/ml (Histamine Degrading Unit/ml) were considered normal while values below 80 HDU/ml were considered DAO deficient. One HDU corresponds to the DAO activity that degrades 1 pmol/ml of histamine.

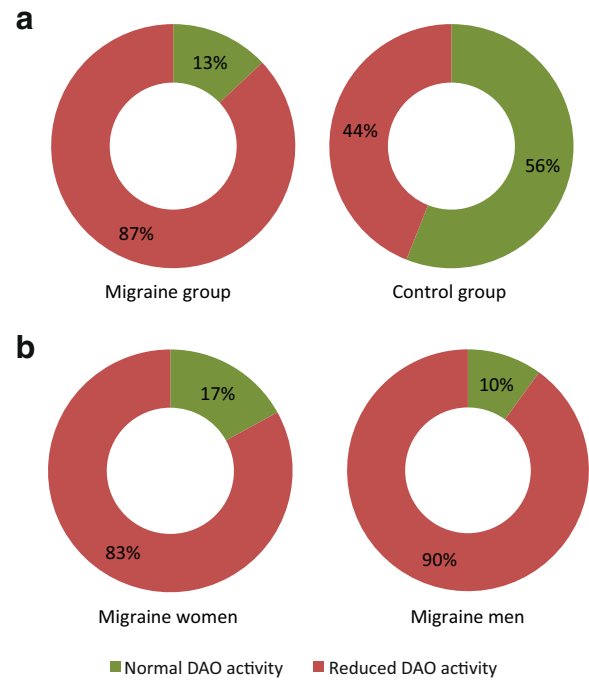
**Statistical analysis**

Data distribution and statistical analysis was performed using SPSS for Windows, version 22 (Chicago, IL). Data distribution was obtained using the Kolmogorov-Smirnov test. As data were not normally distributed, Mann-Whitney test was used to compare DAO activity between both groups. Probability values of  $p < 0.05$  were accepted as significant.

**Results**

The prevalence of DAO deficiency (<80 HDU/ml) assessed in migraine patients and individuals without clinical criteria for migraine as control group is shown in Fig. 1a. A high prevalence of DAO deficiency was observed in the migraine group with 87% of subjects with this enzymatic deficiency in comparison to 44% in the control group. Within the migraine group, the percentage of individuals that showed normal DAO activity levels was 13%. Figure 1b shows the proportion of DAO deficiency in the migraine group by gender. Although the number of women included in the study was higher than men, DAO deficiency was similar in both cases (86 and 90%).

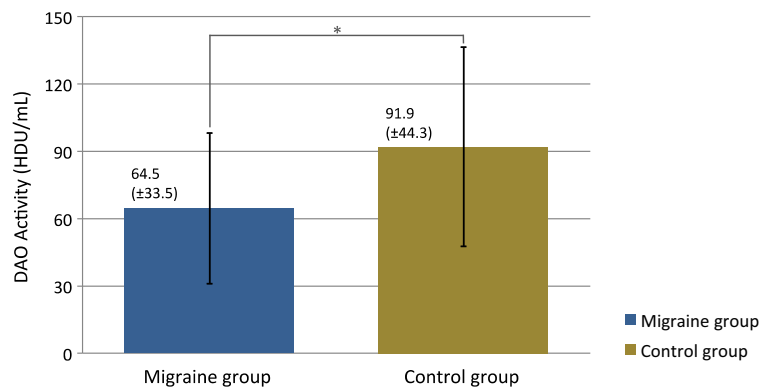
Figure 2 shows the mean DAO activity ( $\pm$ SD) obtained for both study groups. Mean DAO activity in migraine

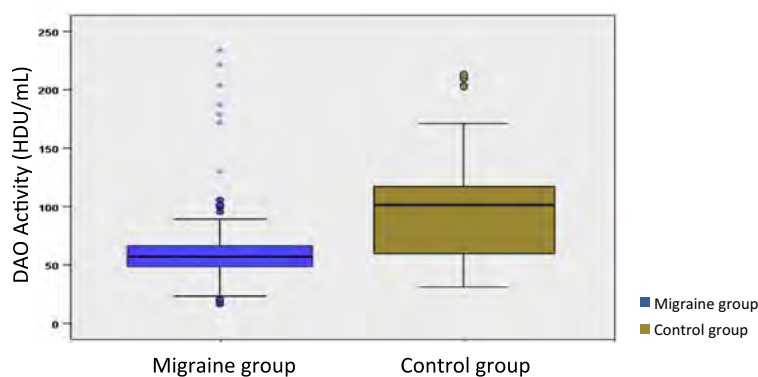


**Fig. 1** Percentage of individuals with deficiency (<80 HDU/ml, red) and normal (>80 HDU/ml, green) DAO activity in both study groups (a) and depending on the gender in the migraine group (b)

population was  $64.5 \pm 33.5$  HDU/ml, being significantly lower (Mann-Whitney U value = 2090.5, Wilcoxon W value = 11,001.5,  $p < 0.0001$ ) than that obtained from control volunteers ( $91.9 \pm 44.3$  HDU/ml). Additionally, Fig. 3 graphically shows the distribution of DAO activity values in both groups. It seems important to highlight that the variability of DAO activity values observed in migraine patients is low, with 50% of cases comprised from 49.5 to 67.1 HDU/ml (percentile 25 and percentile 75, respectively). However, in this group, some extremely high values, statistically considered as outliers, were recorded, reaching DAO activity values close to 250 HDU/ml. On the other hand, greater variability was found in DAO activity values from control individuals. For this group, the interquartile range, calculated as the difference between percentile 75 (118.5 HDU/ml) and percentile 25

**Fig. 2** DAO activity (mean  $\pm$  SD) in migraine patients and individuals without clinical criteria for migraine as control group. A Mann-Whitney test was applied to compare DAO activity in both groups,  $*p < 0.0001$





**Fig. 3** Distribution of DAO activity in migraine patients and individuals without clinical criteria for migraine as control group. The *bottom* and *top* of the box (interquartile range) are the percentile 25 and the percentile 75, respectively. *Central line* represents the median. *Lines* extending

vertically from the boxes (*whiskers*) indicate variability outside the interquartile range. Values statistically considered as outliers are plotted as *circles* (atypical value) or *asterisks* (extremely atypical value)

(59.80 HDU/ml), was 58.7 HDU/ml, threefold higher than the interquartile range obtained for migraine group (17.6 HDU/ml). Similarly to the migraine group, some atypically high DAO activity values were found, with maximum levels up to 211 HDU/ml.

## Discussion

Headache has been reported as one of the most prevalent and disabling disturbances associated with an excess of histamine based on a deficit of DAO [17]. Back in 1993, Wantke et al. [29] described that headaches of 33 out of 45 patients decreased in frequency, duration and intensity after 4 weeks of avoiding histamine-rich foods, such as fish, cheese, hard cured sausages, pickled cabbage and alcoholic beverages. These authors hypothesised that a diminished histamine degradation based on a deficiency of DAO could be the cause of this food intolerance. Recently, a relationship between functional SNPs in the DAO gene and the risk for migraine has been proposed. García-Martín et al. [7] studied the frequency of four different genotypes and allelic variants in 197 patients with migraine and 245 healthy controls from Spain. The DAO SNP rs10156191, associated with decreased DAO enzyme activity, seemed to be more frequent in the migraine population. In the same vein, another study performed by Meza-Velázquez et al. [20] also found that a mutant DAO SNP was significantly more frequent in a group of women with migraine than in the control group. Despite that published studies seem to indicate that DAO deficit could be one of the triggers for headaches, data about serum DAO activity levels in affected populations would be important to support this association.

In this work, serum DAO activity was studied in patients diagnosed with migraine in comparison with a non-migrainous population. The prevalence of DAO deficiency within migraine patients was elevated, finding that 87% of these individuals had serum DAO levels below the cut-off

value of 80 HDU/ml (Fig. 1a). DAO deficiency was not found to be higher in women (Fig. 1b) despite that several authors have associated DAO levels with some female sex hormonal changes [11, 14]. Moreover, the mean value of DAO activity in the migraine group was significantly lower than that obtained in the control group (Fig. 2). These results point out that this enzymatic deficit could be related to the onset of migraine. On the other hand, the fact that 13% of migraine patients showed normal DAO activity levels evidenced that this enzymatic deficiency could be one of the triggers of migraine but not the single trigger responsible for this pathology with multifactorial aetiology.

In the control group, 44% of volunteers showed DAO enzyme deficiency but absence of migraine. As was previously mentioned, impaired intestinal histamine degradation by a deficit of DAO leads to the appearance of multi-faced clinical symptoms, which can coexist in histamine intolerants. In fact, headache is just one of the many symptoms associated with this intolerance. Unfortunately, no other symptoms were recorded in this study and therefore, it cannot be concluded that those individuals were actually asymptomatic for histamine intolerance.

It also has to be stated that DAO activity values found in the control group were more variable than those reported by migraine patients. This wide variability was also observed in the study performed by Manzotti et al. [19], which reported a larger range of DAO activity values for the cohort of healthy controls than in individuals suffering from histamine intolerance.

As in the present work, other clinical studies have been focused in the evaluation of serum DAO activity in specific pathologies (Table 2). In a previous study also focusing on neurological symptomatology, Steinbrecher and Jarisch [25] described that 23 out of 27 potential histamine-intolerant patients suffering from headache (85%) had decreased DAO levels. Furthermore, after 4 weeks of histamine-free diet, a

**Table 2** Summary of the studies that measured serum DAO activity levels in patients with generic symptoms potentially related to histamine intolerance or other specific pathologies

Reference	Pathology	Study subjects	% of DAO deficiency	DAO activity <sup>a</sup>
[22]	Generic symptoms of histamine intolerance	316 patients with clinically suspected histamine intolerance 55 healthy controls	80 22	— —
[19]	Generic symptoms of histamine intolerance	14 patients with clinically suspected histamine intolerance 34 healthy controls	71 —	7.04 39.5
[25]	Headache	35 histamine intolerant patients with headache	85	—
[16]	Atopic eczema	162 patients with atopic eczema 124 patients with symptoms of histamine intolerance but without atopic eczema 85 healthy controls	19 20 0	— — —
[28]	Chronic spontaneous urticaria	55 patients suffering for chronic urticaria and gastrointestinal disturbances	14	17.8
[30]	Atopic dermatitis	58 patients with atopic dermatitis 19 healthy controls	— —	10 14
[2]	Chronic idiopathic urticaria	75 patients with chronic idiopathic urticaria 25 healthy controls	57 40	— —
[9]	Inflammatory bowel diseases	55 patients with Crohn's disease 43 patients with ulcerative colitis	— —	8.5 8.9
[6]	Lactose malabsorption	17 healthy controls 121 patients with lactose malabsorption	— 36	10.3 13.6
[26]	Damage of intestinal mucosa	21 patients with anorexia nervosa restricting type 15 patients with anorexia nervosa binge-eating/purging type 20 healthy controls	— — —	8.2 12.3 12.1
[21]	Damage of intestinal mucosa	20 patients with unresectable metastatic gastric cancer	—	2.4
[8]	Chronic abdominal pain	16 paediatric patients diagnosed with two of more digestive complaints 394 paediatric patients	88 8	— 4.5

<sup>a</sup> Reduced DAO activity <10 U/mL

significant rise in DAO activity was noted and the majority of patients reported a complete remission or improvement in headache frequency.

Considering dermatological symptoms, Maintz et al. [16] evaluated serum DAO activity in patients with atopic eczema in comparison with histamine-intolerant patients without atopic eczema and also with healthy volunteers. No individuals with this enzymatic deficiency were found in the healthy control group. On the contrary, the percentage of patients with DAO deficiency was 19% in atopic eczema group and 20% in histamine intolerant without this dermatological affection. Thus, both a significantly lower mean DAO activity and a higher total number of individuals with a reduced DAO activity was found in atopic eczema patients and histamine intolerant without atopic eczema in comparison with healthy controls. In another study that considered patients with chronic spontaneous urticaria accompanied by gastrointestinal disturbances, a prevalence of DAO deficiency of 44% was observed [28]. Conversely, other studies involving patients with atopic dermatitis and urticaria did not find statistically significant association between a reduced DAO activity and high plasma histamine levels in patients suffering from these skin diseases [2, 30].

In the field of gastrointestinal disorders, Honzawa et al. [9] evaluated the clinical significance of serum DAO activity in 98 patients with inflammatory bowel disease. This study demonstrated that DAO activity was significantly lower in patients with Crohn's disease or ulcerative colitis than in healthy controls, indicating a relationship between DAO levels and intestinal permeability. Furthermore, Enko et al. [6] measured serum DAO levels in 121 patients with lactose malabsorption, finding that 36.4% of this cohort showed a deficiency in this enzyme. Additionally, it was observed that individuals with lactose malabsorption and deficit of DAO tended to report more gastrointestinal symptoms during the lactose hydrogen breath test than those with normal DAO activity. Other authors concluded that low serum DAO activity levels could act as indicator of intestinal mucosal disturbances in patients with anorexia nervosa [26] or under chemotherapy treatment [21].

In clinical studies dealing with paediatric populations, an observational retrospective study performed by Rosell-Camps et al. [23] that involved 16 children with abdominal pain, chronic diarrhoea and vomiting found a direct relation between reduced serum DAO levels and these digestive complaints. Concretely, 88% of these paediatric patients showed DAO deficiency. More recently, in an observational study performed by Hoffmann et al. [8] in 394 children with chronic abdominal pain, only 8% showed DAO activity levels under the normal threshold.

The high prevalence of DAO deficiency in migraine patients found in the current study (87%) coincides with that described by Steinbrecher and Jarisch [25] in patients with headache (85%) and by Rosell-Camps et al. [23] in paediatric

patients with digestive complaints (88%). Moreover, these percentages are in good agreement with those described by Mušič et al. [22] and Manzotti et al. [19], which considered patients clinically suspected as histamine intolerant with diverse coexisting symptoms (Table 2). However, other studies addressing different specific pathologies, such as atopic eczema, chronic urticaria, lactose malabsorption and chronic abdominal pain, reported lower percentages of DAO deficit, with values ranging between 8 and 57% [2, 6, 8, 16, 28].

In view of the results of this study, it can be concluded that DAO deficiency is more prevalent in migraine patients than in non-migranous individuals. More studies are needed to better establish the cut off value of DAO activity to allow not only a more accurate diagnosis of histamine intolerance but also to potentially become an additional diagnosis criterion for migraine. Likewise, further research is necessary to reasonably explain the variability found in serum DAO activity levels.

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#### Compliance with ethical standards

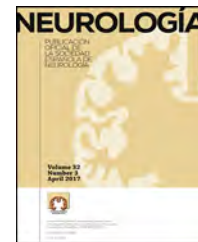
**Conflict of interest** The authors declare that they have no conflict of interest.

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**Association of diamine oxidase and histamine N-methyltransferase polymorphisms with presence of migraine in a group of Mexican mothers of children with allergies.** R. Meza-Velázquez, F. López-Márquez, S. Espinosa-Padilla, M. Rivera-Guillen, J. Ávila-Hernández y M. Rosales-González, **2016** Sociedad Española de Neurología. Elsevier España, S.L.U.



## ORIGINAL ARTICLE

# Association of diamine oxidase and histamine N-methyltransferase polymorphisms with presence of migraine in a group of Mexican mothers of children with allergies<sup>☆</sup>



R. Meza-Velázquez<sup>a,d</sup>, F. López-Márquez<sup>b</sup>, S. Espinosa-Padilla<sup>c</sup>, M. Rivera-Guillen<sup>b,d</sup>, J. Ávila-Hernández<sup>b</sup>, M. Rosales-González<sup>a,\*</sup>

<sup>a</sup> Facultad de Medicina, Departamento de Investigación, Universidad Juárez del Estado de Durango, Campus Gómez Palacio, Durango, Mexico

<sup>b</sup> Facultad de Medicina, Centro de Investigación Biomédica, Universidad Autónoma de Coahuila, Torreón, Coahuila, Mexico

<sup>c</sup> Instituto Nacional de Pediatría, Departamento de Inmunodeficiencias, México, DF, Mexico

<sup>d</sup> Secretaría de Salud, Centro de Atención a Metales Pesados, Torreón, Coahuila, Mexico

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Diamine oxidase;  
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Histamine  
N-methyltransferase;  
ABP1

### Abstract

**Background:** Low histamine metabolism has been suggested to play a role in the pathogenesis of allergy and migraine. We investigated the possible association between 2 single-nucleotide polymorphisms (SNP), C314T *HNMT* and C2029G *DAO*, and the presence and severity of migraine and migraine-related disability.

**Materials and methods:** We studied the frequency of C314T *HNMT* and C2029G *DAO* allelic variants in 162 mothers of children with allergies (80 with migraine and 82 without) using a TaqMan-based qPCR Assay and a case–control model. We conducted a logistic regression analysis to examine the association between migraine and the allelic and haplotype variants.

**Results:** Mutant C2029G *DAO* SNP was found significantly more frequently in the group of women with migraine than in controls (OR, 1.6; 95% CI, 1.1–2.1). No significant differences were found in frequencies of genotypes or alleles in the case of C314T *HNMT* SNP. Both mutated alleles were associated with migraine-related disability. Coexistence of alleles for both SNPs (haplotypes) showed a strong association with migraine. Haplotypes containing both mutated alleles (either heterozygous or homozygous) were very strongly associated with MIDAS grade IV

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\* Corresponding author.

E-mail address: [manuel.rosales.9@hotmail.com](mailto:manuel.rosales.9@hotmail.com) (M. Rosales-González).

**PALABRAS CLAVE**

Migraña;  
 Histamina;  
 Polimorfismos;  
 Histamina  
 N-metiltransferasa;  
 Diaminoxidasa;  
 ABP1

migraine (OR, 45.0; 95% CI, 5.2–358). This suggests that mutant alleles of C314T for histamine N-methyltransferase (HNMT) and C2029G for diamine oxidase (DAO) polymorphisms may interact in a way that increases the risk and impact of migraine.

**Conclusions:** We suggest a synergistic association between HNMT and DAO functional polymorphisms and migraine; this hypothesis must be further confirmed by larger studies. However, the characteristics and ethnic differences between analysed populations should be considered when interpreting the results.

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### Asociación de polimorfismos de diaminoxidasa e histamina N metiltransferasa con la presencia, discapacidad y severidad de migraña en un grupo de madres mexicanas de niños alérgicos

**Resumen**

**Introducción:** Se ha sugerido que una degradación disminuida de histamina puede contribuir en la patogénesis de migraña y alergia. Este trabajo investiga una posible asociación entre 2 polimorfismos de un solo nucleótido (SNP) de 2 enzimas que degradan histamina, C314T para la histamina N-metil-transferasa (HNMT) y C2029G para diaminoxidasa (DAO), con la presencia, discapacidad y severidad de la migraña.

**Material y métodos:** Se reclutó a 162 madres de niños alérgicos (80 con migraña y 82 sin migraña) determinando las variantes alélicas por PRC tiempo real usando un modelo de casos y controles. Mediante regresión logística se determinaron las OR para los genotipos y haplotipos.

**Resultados:** El alelo mutado G para DAO fue significativamente más frecuente en el grupo de mujeres migrañosas que en los controles (OR = 1,6; IC del 95% = 1,1-2,1). No encontramos diferencias significativas para el alelo mutado T de la HNMT. Ambos alelos mutados estuvieron asociados a la discapacidad causada por la migraña. La coexistencia de ambas mutaciones (haplotipos) mostró una fuerte asociación con migraña. Los haplotipos que tenían ambos alelos mutados (ya sea como homocigotos o heterocigotos) estuvieron fuertemente asociados a la discapacidad por migraña grado IV (OR = 45,0, IC del 95% = 5,2-358). Esto sugiere que los alelos mutados T para HNMT y G para DAO pueden interactuar incrementando el riesgo y el impacto de la migraña.

**Conclusiones:** Se sugiere una asociación sinérgica de polimorfismos de HNMT y DAO con migraña el cual debe ser confirmado en futuros estudios. La interpretación debe tomar en cuenta las características étnicas de la población estudiada.

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**Introduction**

Migraine is considered a recurrent neurovascular headache disorder with varying clinical phenotypes.<sup>1,2</sup> The most common types of migraine, migraine with and without aura, are characterised by presence of recurrent headache lasting 4 to 72 hours, which is exacerbated by physical exercise and associated with nausea, photophobia, phonophobia, and neurological symptoms.<sup>3</sup> Prevalence of migraine is estimated at 4% before puberty and increases to 25% in women of childbearing age. The disorder has considerable personal, social, and economic impact.<sup>4</sup> The exact causes and pathophysiological mechanisms of migraine are still to be determined.<sup>2</sup>

According to several studies, some rare and common forms of migraine have a genetic basis.<sup>5,6</sup> For example, several genes with an involvement in familial hemiplegic

migraine have been identified.<sup>5</sup> In contrast, the most prevalent types of migraine (migraine with and without aura) are polygenic and the influence of genetic factors is difficult to determine.<sup>7</sup> According to a study by Ducros et al.,<sup>5</sup> nearly half of the studied families with familial hemiplegic migraine displayed mutations in the calcium-channel gene *CACNA1A*, located on chromosome 19, whereas a locus was mapped on chromosome 1 in the other half of the families; the role of these loci in typical migraine is still to be understood. Genetic approaches to migraine with and without aura include linkage analysis and genome-wide association studies.<sup>5,6,8</sup> These techniques have identified susceptibility loci for migraine on chromosomes 19, 4, and X.<sup>5</sup>

Some neurotransmitters have been associated with migraine, the most frequently studied being serotonin and dopamine.<sup>9–11</sup> Three genes related to the serotonergic system have been associated with migraine with and without

aura: the HTR2B and MAO A receptor genes have been associated with susceptibility for migraine without aura, whereas the dopa-decarboxylase receptor gene has been associated with susceptibility for migraine with aura.<sup>10</sup> Furthermore, there is evidence that several single-nucleotide polymorphisms (SNP) in genes of the dopaminergic system play a role in the pathogenesis of migraine. Three SNPs have been found to be associated with migraine with aura: rs2097629 in the dopamine beta-hydroxylase gene, rs7131056 in *DRD2*, and rs40184 in *SLC6A3*.<sup>11</sup>

Histamine is a neurotransmitter and neuromodulator that appears to have a significant involvement in migraine pathogenesis.<sup>12,13</sup> Migraine attacks may be triggered by the ingestion of histamine-rich foods<sup>14</sup>; in fact, headache is a common feature of histaminosis.<sup>15</sup> In migraine patients, headache may be histamine-dose-dependent.<sup>16,17</sup> Histamine is degraded by 2 enzymes: histamine N-methyltransferase (HNMT; EC 2.1.1.8) and diamine oxidase (DAO) or amiloride-binding protein 1 (ABP1; EC 1.4.3.6). HNMT is involved in the degradation of intracellular histamine<sup>18</sup> whereas DAO is secreted and participates in the degradation of extracellular histamine.<sup>18,19</sup>

The genes coding for HNMT and DAO are polymorphic. The *HNMT* gene is located at 2q22.1 and has 8 SNPs; only one of these is non-synonymous and is located in exon 4, causing the amino acid substitution Thr105Ile (rs11558538).<sup>20</sup> This gene has been associated with decreased HNMT activity.<sup>21</sup> Three non-synonymous SNPs have been mapped to the *DAO* gene, located at 7q34-36. SNP rs1049793 has been found to decrease the protein's activity by altering its form through amino acid substitution His645Asp.<sup>22</sup> In light of the above, our study focuses on these 2 SNPs, the *HNMT* Thr105Ile and *DAO* His645Asp polymorphisms, which we will refer to as C314T and C2029G, respectively, to reflect nucleotide substitutions.

These SNPs have been studied in the context of certain histamine-related conditions, mainly allergic diseases. An association between asthma and *HNMT* Thr105Ile was described by Yan et al.,<sup>23</sup> although this has not been observed in subsequent studies.<sup>24,25</sup> Likewise, no association between that SNP and such other histamine-related conditions as ulcerative colitis<sup>26</sup> and gastric ulcer<sup>27</sup> has been found. However, more recent studies have reported a positive correlation between this polymorphism and presence of asthma.<sup>28,29</sup> Furthermore, no association has been found between the C2029G *DAO* polymorphism and increased risk of histamine-related conditions; this polymorphism has, however, been linked to severity of ulcerative colitis symptoms<sup>26</sup> and clinical manifestations of asthma and allergic rhinitis.<sup>30</sup> Only 2 studies have addressed the role of these 2 polymorphisms on migraine pathogenesis, reporting no significant association.<sup>31,32</sup> Our study evaluated the association between the *HNMT* C314T and *DAO* C2029G SNPs, and presence, severity, and associated disability of migraine in a sample of Mexican mothers of children with allergies.

## Material and methods

We conducted a case-control study and analysed data using a logistic regression model. Our study included

162 unrelated adult women aged 20 to 55 years (mean age,  $34.2 \pm 7.1$  years). They all were mothers of allergic children cared for at the paediatric allergology service of the Secretariat of Health of Torreón, in Coahuila, Mexico, between March 2013 and May 2014. Eighty women met the diagnostic criteria for migraine and not for any other type of headache; these women were included in the case group. Migraine was diagnosed by a specialist with the help of a neurologist, using a validated questionnaire and following the diagnostic criteria of the International Headache Society.<sup>3</sup> The control group included 82 women who had not experienced headache for at least one year prior to inclusion in the study.

Cases reported having experienced episodes of pain for a mean of  $7.3 \pm 4.7$  years; 20.9% had a family history of migraine and 58.3% had migraine with aura. The Migraine Disability Assessment (MIDAS) test was used to determine migraine severity and level of disability in the case group.<sup>33</sup> None of the cases were receiving preventive treatment for migraine; they only took analgesics during headache episodes. Controls were unrelated healthy Mexican women who did not experience migraine or other type of headache.

The selected women were invited to participate in the study and signed informed consent forms prior to inclusion. Our study complies with the principles of the Declaration of Helsinki. The study was approved by the research ethics committee of the School of Medicine at Universidad Autónoma de Coahuila, Mexico. The women included and their children participated in another genetic association study conducted by our research group to analyse allergies.

DNA was obtained from peripheral blood (5 mL) using the "salting-out" procedure.<sup>34</sup> Polymorphisms were analysed using real-time polymerase chain reaction (PCR). Genotyping was conducted using TaqMan technology (Applied Biosystems®) to detect the following SNPs: *DAO* rs1049793 (C\_7599774\_10), a non-synonymous SNP causing the His645Asp amino acid substitution, and *HNMT* rs11558538 (C\_11650812\_20), a non-synonymous SNP causing the Thr105Ile amino acid substitution.

Polymorphisms were analysed with an Applied Biosystems® 7300 Real-Time PCR System using a DNA concentration of 8 ng. Cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, then 40 cycles at 95°C for 15 seconds, and 60°C for 90 seconds. Of the 324 samples collected (162 for *HNMT* and 162 for *DAO*), 320 were successfully genotyped for the 2 SNPs analysed in this study (160 for *HNMT* and 160 for *DAO*).

We estimated the proportion of patients with the *HNMT* and *DAO* SNPs in each group. Differences were analysed with the Chi-square test or the Fisher exact test, as appropriate. We estimated the magnitude of the association between migraine and the *HNMT* and *DAO* allelic variants using odds ratios (OR) with 95% confidence intervals (95% CI).

To evaluate whether the *HNMT* and *DAO* SNPs had a synergistic effect on migraine severity or disability, subjects were classified as SNP carriers or non-carriers, considering several haplotypes. We conducted a logistic regression analysis to assess whether these haplotypes were correlated with migraine severity and disability. Associations were expressed using OR with 95% CI. Two-tailed *P*-values  $\leq .05$  were considered statistically significant. Statistical analysis was performed using STATA 11.1® statistical

software. Hardy-Weinberg equilibrium was tested using Arlequin software v2000 (CMPG Zoological Institute, University of Bern, Bern, Switzerland).

## Results

A total of 162 mothers of allergic children were included in our study. Mean age was  $34 \pm 7.1$  years. Eighty had migraine according to the diagnostic criteria of the International Headache Society and 82 were controls. Table 1 summarises the main clinical characteristics of our sample. Presence of a family history of allergies and migraine was significantly more frequent in cases than in controls ( $P < .05$ ).

In the control group, the genotype distribution of the SNPs studied followed the Hardy-Weinberg equilibrium. In the case group, the genotype distribution of the *HNMT* SNP also followed the Hardy-Weinberg equilibrium, while that of the *DAO* polymorphism did not. A significant difference was observed in the genotype distribution of this SNP between mothers with migraine and a healthy Hispanic population ( $P = .001$ ).<sup>35</sup>

Table 2 shows genotype distributions and allele frequencies for *HNMT* and *DAO* in both groups. According to the comparison of *HNMT* allele frequencies, the mutant T allele of the C314T polymorphism was not significantly more frequent in cases than in controls ( $P = .189$ ) (Table 2).

However, the mutant G allele of the C2029G *DAO* polymorphism was significantly more frequent in patients with migraine ( $P = .044$ ). The OR of migraine for carriers of the mutant G allele of *DAO* compared to wild-type homozygous women was 1.6 (95% CI, 1.1–2.1). Likewise, G/G homozygous women were at greater risk of migraine than wild-type homozygous women (OR, 1.9; 95% CI, 1.3–2.5) (Table 2).

The women were classified as carriers and non-carriers of each of the mutations (whether homozygous or heterozygous) to gather data on the effects of each polymorphism on the phenotypes and clinical characteristics of the study population. The *HNMT* and *DAO* polymorphisms showed a similar distribution when the population was subdivided according to presence of aura and mean duration of migraine attacks. Both polymorphisms were positively correlated with high MIDAS scores. Presence of the C allele for the *HNMT* polymorphism was associated with a lower degree of disability due to migraine (OR, 10.0; 95% CI, 2.65–37.6). In contrast, presence of the T allele (in heterozygous individuals) was associated with higher degree of disability (OR, 37.1; 95% CI, 4.37–315.2) (Table 3). The small number of homozygous mutant individuals prevented us from analysing the frequency of migraine-related disability in this subset.

Presence of the C allele for the *DAO* polymorphism was associated with a lower level of disability (OR, 4.41; 95% CI, 1.07–18.12). Likewise, homozygous C/C individuals were observed to display a lower level of disability (OR, 2.6; 95% CI, 1.05–6.54). In contrast, presence of the G allele was a risk factor for severe disability. Homozygous G/G individuals were at a greater risk of severe disability (OR, 17.6; 95% CI, 2.12–145.6) (Table 3).

A logistic regression analysis of the haplotypes revealed a stronger association between these polymorphisms and

migraine. We considered 4 haplotypes (Table 4). Haplotype 1: wild-type alleles in both loci. Haplotype 2: a single mutant allele in the *DAO* locus. Haplotype 3: a single mutant allele in the *HNMT* locus. Haplotype 4: mutant alleles in both loci (whether homozygous or heterozygous). We found significant differences in haplotype frequency between women with and without migraine ( $P = .038$ ). In women with migraine, we observed a strong association between haplotype 1 and grade I disability (OR, 11.5; 95% CI, 1.3–90) and an even stronger association between haplotype 4 and grade IV disability (OR, 45.0; 95% CI, 5.2–558). We could not estimate the OR for grade III and IV disability given haplotypes 1 and 3 since there were no individuals in those categories (Table 4).

## Discussion

Our study included a sample of mothers of allergic children. The prevalence of allergic diseases in these women (35.8%) was higher than in the general population, suggesting a genetic component to these conditions. The frequency of migraine was higher in our sample (49.4%) than in other female populations,<sup>4</sup> which points to an association between allergic diseases and migraine.<sup>36,37</sup> In fact, we found a greater prevalence of allergic diseases in women with migraine (42.9%) than in those without (29.3%) (Table 1). Significant differences were also observed in presence of family history of allergic diseases between women with and without migraine (35% vs 19.5%;  $P = .026$ ), which suggests that allergic diseases have a genetic basis and are associated with migraine, as previous studies have pointed out.<sup>1,38</sup>

It has been suggested that histamine plays a role in migraine pathogenesis, given that the condition is more frequent in patients with allergic diseases<sup>36,37</sup> and plasma histamine levels are significantly higher in patients with migraine.<sup>39,40</sup> Several studies have reported an increase in spontaneous histamine release from leukocytes in patients with migraine compared to controls<sup>39,41</sup>; furthermore, experimental models have suggested that dural mast cell activation may play a role in migraine pathogenesis.<sup>42</sup>

Our sample displayed an extremely high prevalence of the mutant G allele of the C2029G *DAO* polymorphism (59.4%). However, this allele has also been found to be very frequent in the Hispanic population (43.2%). The higher prevalence of the G allele observed in our study may be due to a selection bias, given that all the women included were mothers of allergic children and may therefore be carriers of an allele also linked to allergic disease. Furthermore, we found a significant difference in prevalence of the G allele between women with and without allergic diseases (data not shown). Genotype distribution of the *DAO* polymorphism was in Hardy-Weinberg equilibrium in women without migraine; however, a deviation from Hardy-Weinberg equilibrium was observed in the subset of women with migraine, which suggests that the 2 populations are different.

The frequency of the mutant T allele in the *HNMT* locus was 15.3% in our sample, compared to 10.4% in the healthy Hispanic population.<sup>43</sup> The genotype distribution of this polymorphism was in Hardy-Weinberg equilibrium in the groups with and without migraine. In addition to this, no

**Table 1** Population characteristics.

Characteristics	Total N=162	Migraine (cases, n=80)	No migraine (controls, n=82)	P
Age, years (mean ± SD)	34.0 ± 7.1	33.8 ± 7.1	34.3 ± 8.1	.718
Allergic diseases (%)	58 (35.8)	34 (42.5)	24 (29.3)	.079
Family history of migraine (%)	34 (20.9)	22 (27.5)	12 (17.6)	.044
Family history of allergic diseases (%)	44 (27.2)	28 (35.0)	16 (19.5)	.026

All participants were mothers of allergic children. A family history of allergic diseases and migraine was reported by participants themselves in all cases. All participants were consecutively selected and unrelated. Data were compared using the Chi-square test.

**Table 2** Genotype and allele frequency distribution for the C314T *HNMT* and C2029G *DAO* SNPs in cases and controls.

SNP	Migraine			No migraine			OR	95% CI
	n	%	95% CI	n	%	95% CI		
<b>C314T <i>HNMT</i></b>								
<i>Genotype</i>								
C/C	52	66.7	60.1–74.9	62	75.6	68.8–81.4	0.2	0.04–1.32
C/T	24	30.8	24.9–38.5	19	23.2	16.5–29.9	1.5	0.75–2.19
T/T	2	3.6	1.2–4.7	1	1.2	0.3–4.4	2.1	0.35–4.40
<i>Allele</i>								
C	128	82.1	76.0–87.2	143	87.2	81.8–92.4	1.5	0.88–2.18
T	28	17.9	11.9–23.8	21	12.8	7.5–18.1		
<b>C2029G <i>DAO</i></b>								
<i>Genotype</i>								
C/C	11	13.8	8.3–19.0	16	20.0	13.9–26.2	0.7	0.20–1.40
C/G	34	42.4	34.6–49.9	41	51.3	43.6–59.5	0.8	0.10–1.40
G/G	35	43.8	36.0–51.5	23	28.7	21.6–36.0	<b>1.9</b>	<b>1.30–2.50</b>
<i>Allele</i>								
C	56	35.0	76.0–88.2	73	45.6	37.8–53.4	1.6	1.10–2.10
G	104	65.0	11.8–24.0	87	54.4	46.7–62.2		

OR values were obtained using logistic regression. Statistically significant values are shown in bold.

significant differences in genotype distribution were found between patients and controls; however, the small size of our sample and the low frequency of the mutant allele may have contributed to these findings. Our results did not change after adjusting for age, disease duration, presence of aura, or consumption of histamine-rich foods. However, the logistic regression analysis revealed a positive correlation between presence of the mutant T allele and presence of migraine-related disability. The homozygous wild-type C/C genotype was associated with grade I migraine-related disability (OR, 10.0; 95% CI, 2.65–37.6) and was found to have a powerful protective effect against grade IV disability (OR, 0.03; 95% CI, 0.01–0.25). Likewise, presence of the T allele in heterozygous women was a risk factor for grade IV disability (OR, 37.1; 95% CI, 4.37–315.2).

Regarding the *DAO* polymorphism, the frequency of the mutant G allele was significantly higher in women with migraine than in controls (65% vs 54.4%;  $P = .044$ ) (Table 2). According to the logistic regression analysis, the C/C genotype was associated with grade I disability (OR, 4.4; 95% CI, 1.07–18.12); G/G homozygous individuals were at greater risk of grade IV disability (OR, 17.6;  $P = .008$ ). The association between these SNPs and symptom severity has previously been reported for other histamine-related conditions.<sup>26,30</sup> Severity of migraine, rated on a 0–10 scale on MIDAS

question B, was moderately correlated ( $r = 0.426$ ) with migraine-related disability (first 5 MIDAS items); however, no association was found between alleles/genotypes and severity of migraine. Menon et al.<sup>44</sup> recently observed an association between the A118G polymorphism of the  $\mu$ -opioid receptor gene and severity of migraine using MIDAS question B exclusively; however, our study only found an association between the SNPs studied and the grade of migraine-related disability.

Co-presence of alleles for both SNPs (haplotypes) showed a strong association with presence of migraine. Haplotype distribution was significantly different in women with and without migraine. Haplotype 1 (wild-type alleles in both loci) was strongly associated with grade I disability (OR, 11.5; 95% CI, 1.3–90), whereas haplotype 4 (mutant alleles in both loci) was strongly associated with grade IV disability (OR, 45.0; 95% CI, 5.2–358). The above suggests that mutant C314T *HNMT* and C2029G *DAO* alleles may interact, increasing patients' degree of migraine-related disability.

The main limitation of our study was its small sample size. We feel, however, that there is sufficient evidence to support the hypothesis of a synergistic effect of both SNPs on migraine, at least in mothers of allergic children, given that type II errors are much more likely to occur than type I errors in studies with small sample sizes. Likewise, our

**Table 3** Association between migraine-related disability and *HNMT* C314T and *DAO* C2029G SNP genotypes.

SNP	Migraine-related disability	OR	95% CI
<b><i>HNMT</i> C314T</b>			
<i>Genotype</i>			
C/C	Grade I	10.00	2.65–37.6
	Grade II	2.13	0.63–7.25
	Grade III	0.34	0.10–1.13
	Grade IV	0.03	0.01–0.25
C/T	Grade I	0.11	0.03–0.41
	Grade II	0.51	0.14–1.73
	Grade III	2.19	0.647–7.41
	Grade IV	<b>37.10</b>	<b>4.37–315.2</b>
T/T	All grades	ND	
<b><i>C2029G</i> <i>DAO</i></b>			
<i>Genotype</i>			
C/C	Grade I	4.41	1.07–18.12
	Grade II	0.58	0.12–2.95
	Grades III and IV	ND	
C/G	Grade I	2.6	1.05–6.54
	Grade II	0.78	0.28–2.16
	Grade III	1.19	0.36–3.93
	Grade IV	0.10	0.02–0.89
G/G	Grade I	0.17	0.06–0.46
	Grade II	1.60	0.60–4.36
	Grade III	1.63	0.49–5.36
	Grade IV	17.6	2.12–145.6

OR values were obtained using logistic regression.

ND: not determined/analysed due to the small number of patients in this category.

**Table 4** Association between haplotypes and migraine-related disability.

Locus	Haplotype	Hap 1	Hap 2	Hap 3	Hap 4
	<i>HNMT</i>	C	C	T	T
	<i>DAO</i>	C	G	C	G
Disability	<i>n</i> (%)	8 (10.3)	44 (57.1)	3 (3.9)	22 (28.6)
Grade I	OR	11.5	2.5	0.6	0.1
	95% CI	(1.3–90)	(0.9–6.5)	(0.06–7.5)	(0.02–0.36)
Grade II	OR	0.4	2.6	1.5	0.4
	95% CI	(0.1–1.5)	(0.83–8.2)	(0.13–18.1)	(0.1–1.5)
Grade III	OR	ND	0.6	ND	3.8
	95% CI	ND	(0.18–1.9)	ND	(1.1–13.1)
Grade IV	OR	ND	0.1	ND	<b>45</b>
	95% CI	ND	(0.02–0.44)	ND	(5.2–358)

For *HNMT*, C is the wild-type allele and T is the mutant allele. For *DAO*, C is the wild-type allele and G is the mutant allele. Haplotype 1 has only wild-type alleles in both loci, haplotype 2 has a mutant allele in the *DAO* locus, haplotype 3 has a mutant allele in the *HNMT* locus, and haplotype 4 has mutant alleles in both loci (whether homozygous or heterozygous). Migraine-related disability was calculated using the MIDAS test. OR values were obtained using logistic regression.

ND: not determined (no participants were included in these categories).

results should be interpreted with caution due to the possibility of selection bias. However, this type of bias could also result in a greater frequency of the risk alleles in our control group; despite this consideration, our study revealed significant differences in genotype distribution between cases and controls.

In conclusion, we present a synergistic association between the C314T *HNMT* and the C2029G *DAO* polymorphisms and the presence of migraine and migraine-related disability; further studies with greater sample sizes are

necessary to confirm this association. The characteristics of the populations studied and the ethnic differences between them should be considered when analysing the results. Furthermore, interactions with other *HNMT* and *DAO* SNPs and other SNPs affecting histamine metabolism (histidine decarboxylase, histamine receptors, etc.) may have an impact on migraine, as recently seen in a study reporting an association between the *PTX3* gene rs3816527 polymorphism with susceptibility to migraine in male patients.<sup>45</sup>

## Conflicts of interest

The authors have no conflicts of interest to declare.

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**Diamine Oxidase rs10156191 and rs2052129 Variants Are Associated With the Risk for Migraine.** Elena García-Martín, MD, PhD; Carmen Martínez, MD, PhD; Mercedes Serrador, MD, PhD; Hortensia Alonso-Navarro, MD, PhD; Pedro Ayuso, MD; Francisco Navacerrada, MD; José A. G. Agúndez MD, PhD; Félix Javier Jiménez-Jiménez, MD, PhD. **2015** Headache, January, American Headache Society, ISSN 0017-8748

## Research Submission

# ***Diamine Oxidase* rs10156191 and rs2052129 Variants Are Associated With the Risk for Migraine**

Elena García-Martín, MD, PhD; Carmen Martínez, MD, PhD; Mercedes Serrador, MD, PhD;  
Hortensia Alonso-Navarro, MD, PhD; Pedro Ayuso, MD; Francisco Navacerrada, MD;  
José A. G. Agúndez, MD, PhD; Félix Javier Jiménez-Jiménez, MD, PhD

**Background.**—Histamine has been implicated in the pathogenesis of migraine. We investigated the possible association between functional single nucleotide polymorphisms (SNPs) in the *diamine oxidase* gene (*DAO*; chromosome 7q36.1, involved in histamine metabolism) and the risk for migraine.

**Methods.**—We studied the frequency of the rs2052129, rs10156191, rs1049742, and rs1049793 genotypes and allelic variants in 197 patients with migraine and 245 healthy controls using a *TaqMan*-based qPCR Assay.

**Results.**—The *DAO* SNP rs10156191, which is related to decreased *DAO* enzyme activity, is associated with the risk of developing migraine, particularly in women. The odds ratio (OR) for the defect allele positivity is 1.61 (95% confidence interval 1.31-2.37) for overall migraine patients and 2.08 (1.29-3.36) for women suffering from migraine. The association was not influenced by confounders such as the age at onset, the presence of aura, positivity of alcohol as a triggering factor, positive family history of aura, or family history of allergy. Multiple regression analyses did not confirm association with the rest of genetic factors.

**Conclusion.**—Our findings, which should be framed as hypothesis generating, suggest that *DAO* genotypes and allelic variants are associated with the risk for migraine in Caucasian Spanish people, especially in women.

**Key words:** migraine, genetics, histamine, *diamine oxidase* gene, risk factor, biomarker

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From the Department of Biochemistry and Molecular Biology, University of Extremadura, Cáceres, Spain (E. García-Martín and P. Ayuso); Red de Investigación de reacciones adversas a alérgenos y fármacos, Instituto de Salud Carlos III, Madrid, Spain (E. García-Martín, C. Martínez, P. Ayuso, and J.A.G. Agúndez); Department of Pharmacology, University of Extremadura, Badajoz, Spain (C. Martínez); Department of Family Medicine, Hospital “Príncipe de Asturias,” Universidad de Alcalá, Madrid, Spain (M. Serrador); Section of Neurology, Hospital Universitario del Sureste, Arganda del Rey (Madrid), Spain (H. Alonso-Navarro, F. Navacerrada, and F.J. Jiménez-Jiménez); Department of Medicine-Neurology, Hospital “Príncipe de Asturias,” Universidad de Alcalá, Madrid, Spain (H. Alonso-Navarro, and F.J. Jiménez-Jiménez); Service of Neurology, Hospital “Ramón y Cajal,” Universidad de Alcalá, Madrid, Spain (F. Navacerrada); Department of Pharmacology, University of Extremadura, Cáceres, Spain (J.A.G. Agúndez).

Address all correspondence to E. García-Martín, Department of Biochemistry and Molecular Biology, University of Extremadura, Av de la Universidad S/N, 10071 Cáceres, Spain, email: elenag@unex.es

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Migraine is a frequent disorder (10-18%) with a male:female ratio of 1:2-3. Despite the high positivity of family history (50-70%) and the increased risk for suffering both migraine with aura (MWA) and migraine without aura (MWOA) by the first-degree relatives of patients, migraine genetics is not well known. Only a number of genes (*CACNA1A*, *ATPIA2*, *SCN1A*) have been identified for familial hemiplegic migraine.<sup>1</sup> In recent years, there has been an increasing interest in the possible relationship between genetic polymorphisms and the risk for migraine, mainly (but not exclusively) related with serotonergic and dopaminergic systems, with variable and inconsistent results (a detailed revision of these studies is out of the scope for the present work). These genetic studies include hypothesis-driven candidate gene association studies and well as hypothesis-free genome-wide association studies (GWAS). The findings obtained in GWAS are so far inconclusive. The first migraine GWAS established association between 2 genes, *MTDH* and *PGCP*, and the risk for migraine.<sup>2</sup> This finding was confirmed in 2 replication studies,<sup>3,4</sup> whereas other studies showed lack of association.<sup>5,6</sup> The putative associations of migraine with diverse SNPs have been refined in some studies.<sup>7-9</sup> Ligthart et al<sup>3</sup> reported association of one SNP in *NGFR* gene and the risk for migraine, but they could not replicate this finding in 3 cohorts. Finally, Chasman et al<sup>7</sup> described association between SNPs in the *PRMD16*, *TRPM8*, and *LRP1* genes. Recent studies identified association between an SNP in the *SLC39A12* gene and the risk for migraine<sup>4,5,10</sup> or MWOA<sup>4,11</sup> in different populations, but failed to find association with SNPs in the *GALNT16* gene.<sup>4,5,10,11</sup> In addition, interethnic variability in the associations exists, as *LRP1* was found to be associated with the risk for migraine in Indians,<sup>4</sup> but not in Chinese<sup>10,11</sup> or Swedish individuals.<sup>5</sup>

Regarding genetic studies based on mechanistic hypothesis, histamine seems to be a plausible candidate involve in the etiology or in the clinical presentation of migraine. Histamine has been implicated in the pathogenesis of migraine headaches in several ways, as summarized in Table 1. Krajewska and Rydzewski<sup>12</sup> reported increased serum DAO activity in 28 patients with migraine (that was even higher

**Table 1.—Clinical, Epidemiological, Biochemical, Experimental, and Pharmacological Data on the Possible Relationship Between Histamine and Migraine**

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*Clinical-epidemiological data*

Higher frequency of frequency of migraine in patients with allergic (histamine-driven) diseases.<sup>35,36</sup>

*Biochemical data*

Increased plasma/serum histamine levels in patients with migraine, both during headache and symptom-free periods.<sup>37-40</sup>

Increased plasma and cerebrospinal fluid levels of histidine (the amino acid precursor of histamine) in patients with migraine during attacks, in comparison with controls.<sup>40</sup>

Increased spontaneous histamine release by leukocytes of migraine patients compared with controls.<sup>39,41-44</sup>

*Experimental data*

Relationship between the activation of dural mast cells, rich in histamine, and migraine pathogenesis shown in experimental models.<sup>45</sup>

*Pharmacological data*

Intravenous infusion of histamine precipitates immediate and delayed headache in patients with tension-type headache and in migraine patients (more severe and pulsatile in the migraine group), but not in controls without headache. This pharmacological effect is most likely through activation of H<sub>1</sub> receptors since pretreatment with histamine HRH1 antagonists, but not with nitric oxide synthase inhibitors, abolishes both immediate and delayed headache induced by intravenous infusion of histamine.<sup>46,47</sup> HRH2 antagonists are much less effective than HRH1 antagonists in abolishing the headache, but they are significantly better than placebo.<sup>46</sup>

N-alpha-methyl-histamine (HRH3 agonist and main metabolite of histamine via histamine N-methyl-transferase) administered subcutaneously has shown efficacy in the prophylaxis of migraine in double-blind, placebo-controlled studies.<sup>48</sup>

Subcutaneous histamine has shown similar efficacy to botulinum toxin type A in migraine prophylaxis in a randomized, double-blind study,<sup>49</sup> and has been considered as probably effective for migraine prevention (level B) in evidence-based guidelines.<sup>23</sup>

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during migraine attacks) compared with 19 controls. In contrast with these findings, a Spanish group claimed, in nonscientific newspapers, that 90% of patients with migraine show DAO deficiency, which could be improved with the ingestion of a DAO capsule before the meals, leading to migraine improvement (<http://www.lavanguardia.com/salud/2013/03/06/54369041093/cientificos-demuestran-que-una-enzima-sirve-para-prevenir-el-90-de-migranas.html>) Histamine acts through four metabotropic histamine receptors, which are all G-protein-coupled (GPCR)

(HRH1, HRH2, HRH3, and HRH4) transducer extracellular signals via Gq, Gs, and Gi/o proteins, respectively. In general, histamine modulates inflammatory and allergic responses via HRH1, gastric acid secretion through HRH2, neurotransmitter release in the central nervous system (CNS) via HRH3, and chemotaxis and inflammatory mediators release via HRH4.<sup>13,14</sup> The brain stores and releases histamine from mast cells and histaminergic neurons of the tuberomammillary nucleus of the posterior basal hypothalamus. Histaminergic fibers project widely to most regions of the CNS, including thalamus, hippocampus, striatum, amygdale, and cerebral cortex.<sup>14-17</sup>

Histamine is synthesized by decarboxylation of its precursor histidine by the enzyme histidine-decarboxylase (E.C. 4.1.1.22), and degraded through 2 enzymes, histamine N-methyltransferase (HNMT, EC. 2.1.1.8) responsible for inactivating histamine in the brain and diamine oxidase (DAO, EC 1.4.3.6), which is responsible for scavenging extracellular histamine after mediator release.<sup>17-19</sup> DAO activity is expressed in peripheral tissues, mainly in the kidney and colon, and in the thymus, and placenta as well.<sup>16</sup> DAO enzyme is codified by the human *amiloride-binding protein 1* gene (also named as *ABPI*, *ABP*, *diamine oxidase DAO* or *DAO1* gene) (chromosome 7q36.1, gene identity 26, MIM 104610). Three common nonsynonymous single nucleotide polymorphisms (SNPs), which bring about 3 amino acid substitutions, namely Thr16Met (rs10156191), Ser332Phe (rs1049742), and His645Asp (rs1049793), have been identified in Caucasian individuals. The functional effects of these SNPs in DAO enzyme activity have been studied in detail.<sup>20,21</sup> Recently, an additional SNP rs2052129 (G4586T), located in the gene promoter, and which seems to cause decreased transcriptional activity, has been described.<sup>22</sup> Individuals carrying rs1049793,<sup>20</sup> rs10156191,<sup>20</sup> and rs2052129<sup>22</sup> minor alleles have shown lower serum DAO activity when compared with noncarriers.

The aim of the present study is to investigate the possible association between the functional SNPs rs2052129, rs10156191, rs1049742, and rs1049793 in the *DAO* gene (which are associated with decreased DAO activity) with the risk of developing migraine in Caucasian Spanish people. This is the first case-

control study analyzing the putative role of functional *DAO* SNPs and the risk of developing migraine.

## PATIENTS AND METHODS

**Patients and Controls.**—We studied 197 patients with diagnostic criteria for migraine, and not for other headache types, according with the classification of the International Headache Society<sup>23</sup> (61 men, 136 women, mean age  $37.5 \pm 12.8$  years, mean age at onset of migraine  $16.8 \pm 10.3$  years), and 245 controls (97 men, 148 women, mean age  $38.9 \pm 15.3$  years). Patients were recruited from those who made their first visit or a follow-up visit to the general neurological clinics of 3 hospitals between September 2006 and August 2007 (113 patients began with migraine episodes under age 15 years, 147 had positive family history for migraine, and 98 had MWA). All eligible patients were invited to participate and all of them agreed to do so. These patients participated in other genetic association studies by our group.<sup>24-28</sup>

Controls were healthy unrelated Caucasian Spanish individuals, most of them students or professors from the University of Extremadura, who did not have either personal or familial positive history of migraine and did not suffer from other headache types. Controls matching gender and age with patients in the study were invited to participate. Control individuals under 18 years were not included in the study. Over 80% of control individuals invited to participate agreed to do so.

All the participants gave written informed consent. The work was done according to the principles of the Declaration of Helsinki. The study protocol was approved by the ethics committees of the University Hospitals “Príncipe de Asturias” (Alcalá de Henares, Madrid, Spain) and “Infanta Cristina” (Badajoz, Spain).

**Genotyping.**—Genotyping was performed in genomic DNA obtained from venous blood samples of participants using TaqMan assays (supplied by Life Technologies, Alcobendas, Madrid, Spain) designed to detect the following SNPs: rs2052129 (C\_\_11630976\_1) a promoter gene variant, rs10156191 (C\_\_25593951\_10) a nonsynonymous variant causing the amino acid substitution Thr 16 Met, rs1049742 (C\_\_7599782\_20) a nonsynonymous variant causing

the amino acid substitution Ser 332 Phe, and rs1049793 (C\_\_7599774\_10) a nonsynonymous variant causing the amino acid substitution His 664 Asp. Detection was carried out by real-time PCR (qPCR) by using an Eppendorf realplex thermocycler (Eppendorf Iberica SLU, San Sebastian de los Reyes, Madrid, Spain) using fluorescent probes. The amplification conditions were: after a denaturation time of 10 minutes at 96°C, 45 cycles of 92°C 15 seconds 60°C 90 seconds were carried out. Fluorescence was measured at the end of each cycle and at endpoint. All samples analyzed were successfully genotyped for all SNPs analyzed. All amplification reactions were determined in triplicate. Genotypes were assigned by the gene identification software (RealPlex 2.0, Eppendorf) and by analysis of the reference cycle number for each fluorescence curve, calculated using the CalQplex algorithm (Eppendorf). Laboratory methods were exactly the same for migraine sufferers and controls.

**Statistical Analysis.**—Statistical analyses were performed using the SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The sample size was determined from the allele frequencies reported for South-European Caucasian individuals in the 1000 genomes catalog (<http://browser.1000genomes.org>), with a genetic model analyzing the frequency for the minor allele with an odds ratio (OR) value = 1.5 ( $P = .05$ ), as recommended for pharmacogenomic studies.<sup>29,30</sup> For genotype comparisons, data were adjusted to dominant, recessive, and allelic models. The best fit was obtained with the dominant model for the minor allele, and this model was used to calculate the OR and  $P$  values. Correction for multiple testing was done according to the false discovery rate (FDR) procedure as described elsewhere.<sup>31</sup> Two comparisons were made in the FDR analyses. In the first one, we analyzed the putative role of factors known to influence DAO activity (genotypes and gender) in the risk of developing migraine (24 comparisons including all genotypes and alleles). In the second comparison, we analyzed putative confounders: age at onset, history of migraine, the presence of aura and antecedents of allergy (36 comparisons including all genotypes and alleles). In addition, logistic analyses under the standard additive model including all genotypes plus gender, age at

onset, alcohol as a triggering factor, family history of migraine, family history of allergy, and presence of aura were carried out in a single model. The Hardy–Weinberg equilibrium was confirmed by means of Arlequin software Ver. 2.000 (CMPG Zoological Institute, University of Berne, Berne, Switzerland).

## RESULTS

The frequencies of *DAO* rs2052129, rs10156191, rs1049742, and rs1049793 genotypes and allelic variants were in Hardy–Weinberg's equilibrium, both in migraine patients and control groups.

The frequencies of genotypes carrying the rs10156191T allele were significantly higher, according to the crude  $P$  values, in migraine patients than in controls (Table 2), and the frequency of rs2052129G allele was significantly higher in migraine patients, although the statistical significance disappeared after multiple comparison analyses. The frequency of the rest of the genotypes did not differ significantly between migraine patients and controls (Table 2).

Regarding the possible influence of gender, carrying the rs2052129G in men and carrying rs10156191T allele in women were associated with increased risk for migraine (Table 3) and the statistical significance remained after correction for multiple comparisons. The frequencies of *DAO* rs2052129, rs10156191, rs1049742, and rs1049793 genotypes and allelic variants were not influenced by age at onset of migraine (Table 4), family history of migraine (Table 4), and presence of aura (Table 4). The frequency of carriers of rs10156191 T was higher in migraine patients with previous history of allergic diseases when compared with that of migraine patients without history of allergy, whereas the frequency of the other studied SNPs was not influenced by the previous history of allergy (Table 5).

Mean  $\pm$  standard deviation age at onset for patients with genotypes rs2052129G/G, GT, and TT were, respectively,  $16.5 \pm 10.3$ ,  $17.5 \pm 10.3$ , and  $17.4 \pm 13.3$  years; for patients with genotypes rs10156191C/C/, C/T, and T/T,  $17.3 \pm 10.9$ ,  $16.2 \pm 9.6$ , and  $16.7 \pm 9.9$  years; for patients with genotypes rs1049742C/C, C/T, and T/T,  $17.0 \pm 10.5$ ,  $16.4 \pm 3.5$ , and  $8.0 \pm 0.5$  years; and for patients with genotypes rs1049793C/C, CG, and GG,  $16.9 \pm 9.7$ ,  $18.3 \pm 11.7$  and

**Table 2.—DAO Genotype and Allelic Variants of Patients With Migraine and Healthy Volunteers**

	Migraine Patients (N = 197, 394 Alleles)	Controls (N = 245, 490 Alleles)	Allele Positivity OR (95% CI); P; Pc
<i>Genotypes</i>			
rs2052129 G/G	120 (60.9; 54.1-67.7)	130 (53.1; 46.8-59.3)	
G/T	70 (35.5; 28.8-42.2)	97 (39.6; 33.5-45.7)	0.73 (0.50-1.06); .099; .297
T/T	7 (3.6; 1.0-6.1)	18 (7.3; 4.1-10.6)	
rs10156191 C/C	108 (54.8; 47.9-61.8)	162 (66.1; 60.2-72.0)	
C/T	77 (39.1; 32.3-45.9)	71 (29.0; 23.3-34.7)	1.61 (1.309-2.37); .015; .072
T/T	12 (6.1; 2.8-9.4)	12 (4.9; 2.2-7.6)	
rs1049742 C/C	174 (88.3; 83.8-92.8)	210 (85.7; 81.3-90.1)	
C/T	21 (10.7; 6.4-15.0)	33 (13.5; 9.2-17.7)	0.79 (0.45-1.39); .419; .864
TT	2 (1.0; 0.4 to 2.4)	2 (0.8; 0.3 to 1.9)	
rs1049793 C/C	109 (55.3; 48.4-62.3)	129 (52.7; 46.4-58.9)	
C/G	69 (35.0; 28.4-41.7)	98 (40.0; 33.9-46.1)	0.90 (0.62-1.31); .575; .864
G/G	19 (9.6; 5.5-13.8)	18 (7.3; 4.1-10.6)	
			Allele Frequency Difference OR (95% CI); P; Pc
<i>Alleles</i>			
rs2052129 G	310 (78.7; 74.6-82.7)	357 (72.9; 68.9-76.8)	0.73 (0.53-0.99); .046; .158
rs2052129 T	84 (21.3; 17.3-25.4)	133 (27.1; 23.2-31.1)	
rs10156191 C	293 (74.4; 70.1-78.7)	395 (80.6; 77.1-84.1)	1.43 (1.04-1.97); .026; .104
rs10156191 T	101 (25.6; 21.3-29.9)	95 (19.4; 15.9-22.9)	
rs1049742 C	369 (93.7; 91.2-96.1)	453 (92.4; 90.1-94.8)	0.83 (0.49-1.40); .485; .864
rs1049742 T	25 (6.3; 3.9-8.8)	37 (7.6; 5.2-9.9)	
rs1049793 C	287 (72.8; 68.5-77.2)	356 (72.7; 68.7-76.6)	0.99 (0.74-1.33); .950; .959
rs1049793 G	107 (27.2; 22.8-31.5)	134 (27.3; 23.4-31.3)	

The values in each cell represent: number (percentage) and (95% confidence intervals). Allele positivity compares the presence of the minor allele either in heterozygosity or homozygosity vs the absence of the minor allele. P, crude *P* value; Pc: corrected *P* value according the false discover rate procedure as described within the methods section. CI = confidence interval; OR = odds ratio.

11.5 ± 6.9 years (nonsignificant differences for the comparison of carriers vs noncarriers of variant alleles). Logistic regression including in a single model all genotypes, gender, age at onset, alcohol as a triggering factor, the presence of family history of aura, family history of allergy, and the presence of aura revealed that in this study group, only 2 factors were related to the risk of developing migraine, namely the rs10156191 C/C genotype (*P* = .021), and the rs10156191 C/T genotype (*P* = .040). The rest of putative genetic associations were discarded in the multiple analysis. The association of both rs10156191 genotypes with the risk of developing migraine was influenced by gender (*P* = .047 and *P* = .031 for the

interaction with the C/C and C/T genotypes, respectively). No interaction of age at onset, alcohol as a triggering factor, the presence of family history of aura, family history of allergy, and the presence of aura with the genetic associations identified in this study was observed.

## DISCUSSION

Despite the fact that clinical and experimental data suggest the possible involvement of histamine in the pathogenesis of migraine (Table 1), the possible contribution of genetic polymorphisms related with histamine in the risk of developing migraine is not well established.

**Table 3.—DAO Genotype and Allelic Variants of Patients With Migraine and Healthy Volunteers Distributed by Gender**

Genotypes	Migraine Women (N = 136, 272 Alleles)		Control Women (N = 148, 296 Alleles)		Allele Positivity OR (95% CI); P; Pc		Migraine Men (N = 61, 122 Alleles)		Control Men (N = 97, 194 Alleles)		Allele Positivity OR (95% CI); P; Pc	
rs2052129 G/G	73 (53.7; 45.3-62.1)	79 (53.4; 45.3-61.4)	0.99 (0.62-1.58); .959; .959	47 (77.0; 66.5-87.6)	51 (52.6; 42.6-62.5)	0.33 (0.16-0.68); .002; .036						
G/T	59 (43.4; 35.1-51.7)	58 (39.2; 31.3-47.1)		11 (18.0; 8.4-27.7)	39 (40.2; 30.4-50.0)							
T/T	4 (2.9; 0.1-5.8)	11 (7.4; 3.2-11.7)		3 (4.9; -0.5-10.3)	7 (7.2; 2.1-12.4)							
rs10156191 C/C	66 (48.5; 40.1-56.9)	98 (66.2; 58.6-73.8)	2.08 (1.29-3.36); .003; .036	42 (68.9; 57.2-80.5)	64 (66.0; 56.6-75.4)	0.88 (0.44-1.74); .708; .864						
C/T	62 (45.6; 37.2-54.0)	43 (29.1; 21.7-36.4)		15 (24.6; 13.8-35.4)	28 (28.9; 19.8-37.9)							
T/T	8 (5.9; 1.9-9.8) (2)	7 (4.7; 1.3-8.1)		4 (6.6; 0.3-12.8)	5 (5.2; 0.8-9.6)							
rs1049742 C/C	119 (87.5; 81.9-93.1)	127 (85.8; 80.2-91.4)	0.86 (0.44-1.72); .676; .864	55 (90.2; 82.7-97.6)	83 (85.6; 78.6-92.6)	0.65 (0.23-1.79); .397; .864						
C/T	16 (11.8; 6.3-17.2)	20 (13.5; 8.0-19.0)		5 (8.2; 1.3-15.1)	13 (13.4; 6.6-20.2)							
T/T	1 (0.7; -0.7 to 2.2)	1 (0.7; -0.6 to 2.0)		1 (1.6; -1.5 to 4.8)	1 (1.0; -1.0 to 3.0)							
rs1049793 C/C	75 (55.1; 46.8-63.5)	79 (53.4; 45.3-61.4)	0.93 (0.58-1.49); .765; .874	34 (55.7; 43.3-68.2)	50 (51.5; 41.6-61.5)	0.85 (0.44-1.61); .607; .864						
C/G	53 (39.0; 30.8-47.2)	59 (39.9; 32.0-47.8)		16 (26.2; 15.2-37.3)	39 (40.2; 30.4-50.0)							
G/G	8 (5.9; 1.9-9.8)	10 (6.8; 2.7-10.8)		11 (18.0; 8.4-27.7)	8 (8.2; 2.8-13.7)							

Alleles	Allele Frequency Difference OR (95% CI); P; Pc		Allele Frequency Difference OR (95% CI); P; Pc			
rs2052129 G	205 (75.4; 70.2-80.5)	216 (73.0; 67.9-78.0)	0.88 (0.61-1.29); .515; .864	105 (86.1; 79.9-92.2)	141 (72.7; 66.4-79.0)	0.43 (0.24-0.79); .005; .040
rs2052129 T	67 (24.6; 19.5-29.8)	80 (27.0; 22.0-32.1)		17 (13.9; 7.8-20.1)	53 (27.3; 21.0-33.6)	
rs10156191 C	66 (48.5; 40.1-56.9)	239 (80.7; 76.3-85.2)	1.69 (1.14-2.49); .008; .048	99 (81.1; 74.2-88.1)	156 (80.4; 74.8-86.0)	0.95 (0.54-1.70); .872; .951
rs10156191 T	62 (45.6; 37.2-54.0)	57 (19.3; 14.8-23.7)		23 (18.9; 11.9-25.8)	38 (19.6; 14.0-25.2)	
rs1049742 C	119 (87.5; 81.9-93.1)	274 (92.6; 89.6-95.6)	0.88 (0.46-1.68); .705; .864	115 (94.3; 90.1-98.4)	179 (92.3; 88.5-96.0)	0.73 (0.29-1.84); .498; .864
rs1049742 T	16 (11.8; 6.3-17.2)	22 (7.4; 4.4-10.4)		7 (5.7; 1.6-9.9)	15 (7.7; 4.0-11.5)	
rs1049793 C	203 (74.6; 69.5-79.8)	217 (73.3; 68.3-78.4)	0.93 (0.64-1.36); .720; .864	84 (68.9; 60.6-77.1)	139 (71.6; 65.3-78.0)	1.14 (0.70-1.87); .595; .864
rs1049793 G	69 (25.4; 20.2-30.5)	79 (26.7; 21.6-31.7)		38 (31.1; 22.9-39.4)	55 (28.4; 22.0-34.7)	

The values in each cell represent: number (percentage) and (95% confidence intervals). Allele positivity compares the presence of the minor allele either in heterozygosity or homozygosity vs the absence of the minor allele. P, crude P value; Pc, corrected P value according the false discover rate procedure as described within the methods section. CI = confidence interval; OR = odds ratio.

**Table 4.—DAO Genotype and Allelic Variants of Patients With Migraine Distributed by Age, Family History, and the Presence of Aura**

Genotypes	Age at Onset ≤ 16 Years (N = 113; 226 Alleles)	Age at Onset ≥ 16 Years (N = 84; 168 Alleles)	Allele Positivity OR (95% CI); P; Pc	Positive Family History of Migraine (N = 147, 294 Alleles)	Negative Family History of Migraine (N = 50, 100 Alleles)	Allele Positivity OR (95% CI); P; Pc	Migraine With Aura (N = 98; 196 Alleles)	Migraine Without Aura (N = 99; 198 Alleles)	Allele Positivity OR (95% CI); P; Pc
	rs2052129 G/G	72 (63.7; 54.9-72.6) 37 (32.7; 24.1-41.4)	48 (57.1; 46.6-67.7) 33 (39.3; 28.8-49.7)	0.76 (0.43-1.35); .349; .934	89 (60.5; 52.6-68.4) 53 (36.1; 28.3-43.8)	31 (62.0; 48.5-75.5) 17 (34.0; 20.9-47.1)	1.07 (0.55-2.06); .855; .934	57 (58.2; 48.4-67.9) 37 (37.8; 28.2-47.4)	63 (63.6; 54.2-73.1) 33 (33.3; 24.0-42.6)
T/T	4 (3.5; 0.1-6.9)	3 (3.6; -0.4 to 7.5)		5 (3.4; 0.5-6.3)	2 (4.0; -1.4 to 9.4)		4 (4.1; 0.2-8.0)	3 (3.0; -0.3 to 6.4)	
rs10156191 C/C	63 (55.8; 46.6-64.9) 45 (39.8; 30.8-48.8)	45 (53.6; 42.9-64.2) 32 (38.1; 27.7-48.5)	0.92 (0.52-1.62); .761; .934	82 (55.8; 47.8-63.8) 56 (38.1; 30.2-45.9)	26 (52.0; 38.2-65.8) 21 (42.0; 28.3-55.7)	0.86 (0.45-1.63); .642; .934	49 (50.0; 40.1-59.9) 43 (43.9; 34.1-53.7)	59 (59.6; 49.9-69.3) 34 (34.3; 25.0-43.7)	1.48 (0.84-2.59); .176; .934
T/T	5 (4.4; 0.6-8.2)	7 (8.3; 2.4-14.2)		9 (6.1; 2.2-10.0)	3 (6.0; 0.6-12.6)		6 (6.1; 1.4-10.9)	6 (6.1; 1.4-10.8)	
rs1049742 C/C	99 (87.6; 81.5-93.7) 12 (10.6; 4.9-16.3)	75 (89.3; 82.7-95.9) 9 (10.7; 4.1-17.3)	1.18 (0.48-2.87); .717; .934	130 (88.4; 83.3-93.6) 15 (10.2; 5.3-15.1)	44 (88.0; 79.0-97.0) 6 (12.0; 3.0-21.0)	0.96 (0.36-2.59); .934; .934	87 (88.8; 82.5-95.0) 10 (10.2; 4.2-16.2)	87 (87.9; 81.4-94.3) 11 (11.1; 4.9-17.3)	0.92 (0.38-2.19); .845; .934
T/T	2 (1.8; -0.7 to 4.2)	0 (0.0; 0.0-0.0)		2 (1.4; -0.5 to 3.2)	0 (0.0; 0.0-0.0)		1 (1.0; -1.0 to 3.0)	1 (1.0; -1.0 to 3.0)	
rs1049793 C/C	62 (54.9; 45.7-64.0) 38 (33.6; 24.9-42.3)	47 (56.0; 45.3-66.6) 31 (36.9; 26.6-47.2)	1.05 (0.59-1.84); .880; .934	81 (55.1; 47.1-63.1) 49 (33.3; 25.7-41.0)	28 (56.0; 42.2-69.8) 20 (40.0; 26.4-53.6)	1.04 (0.54-1.98); .912; .934	55 (56.1; 46.3-65.9) 35 (35.7; 26.2-45.2)	54 (54.5; 44.7-64.4) 34 (34.3; 25.0-43.7)	0.94 (0.54-1.65); .824; .934
G/G	13 (11.5; 5.6-17.4)	6 (7.1; 1.6-12.7)		17 (11.6; 6.4-16.7)	2 (4.0; -1.4 to 9.4)		8 (8.2; 2.7-13.6)	11 (11.1; 4.9-17.3)	

Alleles	Allele Frequency Difference OR (95% CI); P; Pc	Allele Frequency Difference OR (95% CI); P; Pc	Allele Frequency Difference OR (95% CI); P; Pc
	rs2052129 G	181 (80.1; 74.9-85.3)	129 (76.8; 70.4-83.2)
rs2052129 T	45 (19.9; 14.7-25.1)	39 (23.2; 16.8-29.6)	
rs10156191 C	171 (75.7; 70.1-81.3)	122 (72.6; 65.9-79.4)	0.85 (0.54-1.35); .494; .934
rs10156191 T	55 (24.3; 18.7-29.9)	46 (27.4; 20.6-34.1)	
rs1049742 C	210 (92.9; 89.6-96.3)	159 (94.6; 91.2-98.0)	1.35 (0.38-3.13); .487; .934
rs1049742 T	16 (7.1; 3.7-10.4)	9 (5.4; 2.0-8.8)	
rs1049793 C	162 (71.7; 65.8-77.6)	125 (74.4; 67.8-81.0)	1.15 (0.73-1.80); .548; .934
rs1049793 G	64 (28.3; 22.4-34.2)	43 (25.6; 19.0-32.2)	

The values in each cell represent: number (percentage) and (95% confidence intervals). Allele positivity compares the presence of the minor allele either in heterozygosity or homozygosity vs the absence of the minor allele. P: crude P value; Pc: corrected P value according the false discover rate procedure as described within the methods section. CI = confidence interval; OR = odds ratio.

**Table 5.—DAO Genotype and Allelic Variants of Patients With Migraine Distributed According to Antecedents of Allergy**

	Antecedents of Allergy (N = 84; 168 Alleles)	No Antecedents (N = 113; 226 Alleles)	Allele Positivity OR (95% CI); P; Pc
<i>Genotypes</i>			
rs2052129 G/G	48 (57.1; 46.6-67.7)	72 (63.7; 54.9-72.6)	
G/T	33 (39.3; 28.8-49.7)	37 (32.7; 24.1-41.4)	1.32 (0.74-2.35); .349; .934
T/T	3 (3.6; -0.4 to 7.5)	4 (3.5; 0.1-6.9)	
rs10156191 C/C	39 (46.4; 35.8-57.1)	69 (61.1; 52.1-70.1)	
C/T	42 (50.0; 39.3-60.7)	35 (31.0; 22.4-39.5)	1.81 (1.02-3.21); .041; .934
T/T	3 (3.6; -0.4-7.5)	9 (8.0; 3.0-13.0)	
rs1049742 C/C	72 (85.7; 78.2-93.2)	102 (90.3; 84.8-95.7)	
C/T	12 (14.3; 6.8-21.8)	9 (8.0; 3.0-13.0)	1.55 (0.65-3.70); .325; .934
TT	0 (0.0; 0.0-0.0)	2 (1.8; -0.7-4.2)	
rs1049793 C/C	45 (53.6; 42.9-64.2)	64 (56.6; 47.5-65.8)	
C/G	31 (36.9; 26.6-47.2)	38 (33.6; 24.9-42.3)	1.13 (0.64-2.00); .668; .934
G/G	8 (9.5; 3.2-15.8)	11 (9.7; 4.3-15.2)	
			Allele Frequency Difference OR (95% CI); P; Pc
<i>Alleles</i>			
rs2052129 G	129 (76.8; 70.4-83.2)	181 (80.1; 74.9-85.3)	1.22 (0.75-1.97); .429; .934
rs2052129 T	39 (23.2; 16.8-29.6)	45 (19.9; 14.7-25.1)	
rs10156191 C	120 (71.4; 64.6-78.3)	173 (76.5; 71.0-82.1)	1.31 (0.83-2.06); .250; .934
rs10156191 T	48 (28.6; 21.7-35.4)	53 (23.5; 17.9-29.0)	
rs1049742 C	156 (92.9; 89.0-96.8)	213 (94.2; 91.2-97.3)	1.26 (0.56-2.84); .575; .934
rs1049742 T	12 (7.1; 3.2-11.0)	13 (5.8; 2.7-8.8)	
rs1049793 C	121 (72.0; 65.2-78.8)	166 (73.5; 67.7-79.2)	1.08 (0.69-1.68); .753; .934
rs1049793 G	47 (28.0; 21.2-34.8)	60 (26.5; 20.8-32.3)	

The values in each cell represent: number (percentage) and (95% confidence intervals). Allele positivity compares the presence of the minor allele either in heterozygosity or homozygosity vs the absence of the minor allele. P, crude *P* value; Pc: corrected *P* value according the false discover rate procedure as described within the methods section.

CI = confidence interval; OR = odds ratio.

Our group, in an association study involving 197 migraine patients and 245 healthy controls, reported lack of association between Thr105Ile SNP (rs1801105) polymorphism in the *HNMT* gene (located at chromosome 2q22.1, gene identity 3176, MIM 605238) and the risk for migraine.

In the present study, we identified association of the risk of developing migraine with the SNP rs10156191 as well as gender. In addition, subgroup analyses suggested that male homozygotes for rs2052129G and women carrying the rs10156191T allele showed increased risk for migraine. However, subgroup analyses are unreliable because of the sample size and therefore these findings should be

interpreted cautiously. The frequency of DAO genotypes and their association with the risk of developing migraine were unrelated with age at onset, alcohol as a triggering factor, the presence of family history of aura, family history of allergy, and the presence of aura.

It is to be noted that the rs10156191 and the rs2052129 SNPs cause altered enzyme activity *in vivo*.<sup>20,22</sup> The allele with the rs10156191 T sequence encodes a variant with the amino acid substitution Met in the position 16, instead of Thr in the wild-type protein. This amino acid substitution reduces the enzyme intrinsic activity, thus decreasing the ability to metabolize circulating histamine.<sup>19,20</sup>

Because the rs10156191 T is more common among migraineurs (Table 2) and particularly in women (Table 3), it is to be expected that the genetic predisposition to a decreased metabolic clearance of circulating histamine may be involved in the development of migraine. The functional effect of the allele rs2052129 T is a decrease in the enzyme expression.<sup>22</sup> Because among migraineurs there is an increase in the frequency of the rs2052129 G allele, our results point against the hypothesis of decreased histamine metabolism. However, a previous study failed to find clinical associations of the rs2052129 T allele in study groups with positive association for rs10156191 T, thus suggesting that these 2 variant alleles may have quantitatively different clinical impact.<sup>32</sup>

It has been shown that gender is a major factor on DAO enzyme activity *in vivo*, with women displaying higher enzyme activity and higher interindividual variability than men,<sup>21</sup> and therefore, it is not surprising that the association of *DAO* SNPs with migraine would show association with gender. Nevertheless, considering the limited sample size in this study, and that the most significant association was observed in women, the findings obtained in this study would require replication to obtain more support to the proposed association. The *DAO* SNPs studied here have been related with other histamine-related disorders such as drug-induced hypersensitivity,<sup>32</sup> rhinitis,<sup>33</sup> or ulcerative colitis.<sup>34</sup> Aside from the SNPs analyzed in the present study, no additional nonsynonymous SNPs have been reported to occur in Caucasian subjects with a significant minor allele frequency in the genes analyzed according to public databases (<http://browser.1000genomes.org>).

## CONCLUSION

In summary, *DAO* alleles related with decreased DAO enzyme activity seem to be associated with migraine risk in Caucasian Spanish people. These findings, however, should be framed as hypothesis generating. Further studies combining genotyping for *DAO* allelic variants with measurement of DAO serum activity in the same migraine patient and control groups are needed.

## STATEMENT OF AUTHORSHIP

### Category 1

#### (a) Conception and Design

Elena García-Martín; José A. G. Agúndez; Félix Javier Jiménez-Jiménez

#### (b) Acquisition of Data

Elena García-Martín; Carmen Martínez; Mercedes Serrador; Hortensia Alonso-Navarro; Pedro Ayuso; Francisco Navacerrada; José A. G. Agúndez; Félix Javier Jiménez-Jiménez

#### (c) Analysis and Interpretation of Data

Elena García-Martín; José A. G. Agúndez; Félix Javier Jiménez-Jiménez

### Category 2

#### (a) Drafting the Manuscript

Elena García-Martín; José A. G. Agúndez; Félix Javier Jiménez-Jiménez

#### (b) Revising It for Intellectual Content

Elena García-Martín; Carmen Martínez; Mercedes Serrador; Hortensia Alonso-Navarro; Pedro Ayuso; Francisco Navacerrada; José A. G. Agúndez; Félix Javier Jiménez-Jiménez

### Category 3

#### (a) Final Approval of the Completed Manuscript

Elena García-Martín; Carmen Martínez; Mercedes Serrador; Hortensia Alonso-Navarro; Pedro Ayuso; Francisco Navacerrada; José A. G. Agúndez; Félix Javier Jiménez-Jiménez

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**Histamine in migraine and brain.**  
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## Review Article

# Histamine in Migraine and Brain

Karl B. Alstadhaug, MD, PhD

**Background.**—Histamine has been studied in both health and disease since the initial description a century ago. With its vasodilative effect, it was suggested early on to be involved in the pathophysiology of migraine. Over the past 25 years, much has been learned about histamine as a neurotransmitter in the central nervous system. The role of this neurotransmitter system in migraine has not been previously reviewed.

**Objective.**—Discuss a potential role of the brain histaminergic system in migraine.

**Methods.**—Unstructured literature search with a no specific hypothesis-driven approach.

**Results.**—There is substantial evidence that systemically given histamine may elicit, maintain, and aggravate headache. The mechanisms for this are not known, and histamines do not penetrate the blood–brain barrier (BBB). However, circulating histamine may influence hypothalamic activity via the circumventricular organs that lack BBB. In the rat, prolonged activation of meningeal nociceptors induced by dural mast cell degranulation has been observed. Subcutaneous injections of N-alpha-methyl histamine, a catabolite of histamine with high affinity to the histamine H3 receptor, probably have some migraine preventive effect. A negative feedback on histamine release from mast cells in proximity to C-fiber endings has been a postulated mechanism.

Most antihistamines have shown to be ineffective as acute medication for migraine. Two centrally acting potent H1 receptor antagonists (cinnarizine and cyproheptadine) have been reported to be efficacious in preventing migraine. However, the proof for this is limited, and their efficacy has been ascribed other actions than the antihistaminergic. In general, lack of specificity and side effects limit the potential use of centrally acting H1 and H2 antagonists.

Brain histamine is synthesized by neurons that are restricted to the posterior basal hypothalamus, more specific to the tuberomammillary nucleus (TMN), and that project practically to the whole central nervous system. The posterior hypothalamus is a suspected *locus in quo* in several primary headaches. Recently, a positron emission tomography study performed in the prodromal phase of migraine attacks supported the idea of initial involvement of this area. In another recent study, the thalamic nuclei receiving trigeminal output was also shown to have direct connections with the ventral TMN. The central histaminergic system plays an important role in the complex sleep–wake cycle, promoting cortical excitability during waking and attention, and it consolidates the wake state. The period of the day, in the evenings and during the night, when there is reduced susceptibility for migraine attacks corresponds with less central histaminergic firing. Activation of both the H3 and the H4 receptor promotes inhibitory actions on neurons. The H3 receptor causes autoinhibition of the histaminergic neurons themselves, and centrally acting H3 receptor agonist prodrugs have shown to both inhibit neurogenic inflammation in dura, to induce sleep, and to produce antinociception. There are no registered ongoing studies on H3 and H4 receptor ligands in migraine.

**Conclusion.**—The role of the central histaminergic system in migraine is largely unexplored, but findings from preclinical research may be linked to several aspects of the disorder. The histaminergic system of the brain may play an important role, especially in the initial phase of an attack, and histamine H3 and H4 receptor ligands may potentially have migraine prophylactic properties. However, the basis for this is still circumstantial, and the evidence is lacking.

**Key words:** histamine, migraine, hypothalamus, pathophysiology, antihistamine, chronobiology

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From the Department of Neurology, Nordland Hospital Trust, Bodø, Norway; Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway.

Address all correspondence to K.B. Alstadhaug, Nordlandssykehuset, Neurology, Prinsens gate 164, Bodø 8011, Norway.

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*Conflict of Interest.* The author reports no conflicts of interest.

**A randomized doubled blinded trial of treatment with DiAmineOxidase (DAO) in patients with migraine and deficit of enzyme's activity.** J. Izquierdo, D. Mon, M. Lorente, L. Soler Singla. Hospital General de Catalunya, Universitat Internacional de Catalunya, Sant Cugat del Vallès, Spain. **2013** XXI World Congress of Neurology. Wien/Austria. Journal of the Neurological Sciences 333

**Objective:** To ascertain the effect of perioperative BP on the development of PDPH in the patients who received surgery under spinal anesthesia.

**Patients and methods:** We evaluated the presence of PDPH in all consecutive 199 patients (122 males, 77 females, age: 15–76 years) who received elective knee surgery under spinal anesthesia between September 2012 and February 2013. The spinal anesthesia was performed by the same anesthesiologist with 25-G Quincke needle. Data regarding previous history of headache, pre- and post-operative BP, highest and lowest BP during operation as well as demographic features were analysed.

**Results:** The overall incidence of PDPH was 9.0%. It was higher in female than in male (15.6% vs 4.9%,  $p = 0.02$ ). Age, history of hypertension or recurrent headache was not different between patients with and without PDPH. The duration of operation or spinal anesthesia was not different between the two groups. BP variables were expressed as pre- and post-operative mean arterial pressure (MAP), the highest and the lowest MAP during operation, and their differences were not different either.

**Conclusion:** PDPH after knee surgery under spinal anesthesia occurred more frequently in female patients, and was not influenced by their perioperative BP states.

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#### Abstract – WCN 2013

No: 1558

Topic: 8 – Headache

#### Memory improvement after spreading depression by NMDA blocker as memory destructor

B. Khodaie, Neuroscience, Shefa Neuroscience Research Center, Tehran, Iran

Spreading depression (SD) is transient neural hyperexcitability followed by depolarization wave, which propagates through the brain and modulate electrical gradient and synaptic activity. Data have shown that SD wave distributes coincidence between neural activity and behavioral activity. Neural activity and electrical potential effect on memory retrieval have been demonstrated. Inhibitory effect of NMDA receptors in SD procedure can control memory impairment caused by SD. However, the negative effect of NMDA receptor blockage on memory has been proven in previous studies. In the present study the effect of NMDA receptors blockage (MK801) used to evaluate its efficiency in subsiding of SD negative influence on memory. Wistar rats (60–80 g) were randomly chosen in 6 groups and (NMDA blocker 0.63–1 mg/kg) were administrated after 3 mol/L KCl injection for induction of repetitive SD in rat. The groups were evaluated by T-maze test and SD groups were compared with control groups, including (NMDA blocker 1–0.63 mg/kg controls) and sham group. T-maze data have showed that repeated SD could significantly alter memory retrieval performance. However, in the second week memory enhancement was induced by SD induction. Repeated SD induction during other weeks indicated impairment in memory. Application of NMDA blocker showed significantly enhanced memory retrieval and could potentially control memory impairment after SD. The studies indicated that NMDA blocker may decrease memory performance, on the other hand the effect of MK801 on inhibition of SD propagation may somehow weaken memory improvement due to its memory destruction effects.

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#### Abstract – WCN 2013

No: 1647

Topic: 8 – Headache

#### Prevalence of migraine among medical students in Kuwait University

J.Y. Al-Hashel<sup>a,b</sup>, S. Muhammad<sup>a,c</sup>, R. Alroughany<sup>d,e</sup>. <sup>a</sup>Neurology, Ibn Sina Hospital; <sup>b</sup>Medicine, Kuwait University, Kuwait, Kuwait; <sup>c</sup>Neurology, Minia University, Minia, Egypt; <sup>d</sup>Neurology, Amiri Hospital; <sup>e</sup>Neurology, Dasman Diabetes Institute, Kuwait, Kuwait

**Background:** Prevalence of migraine among medical students is of particular interest as they are subjected to lots of tests and stresses that may precipitate migraine attacks.

**Objectives:** To determine the prevalence of migraine among medical students in Kuwait University.

**Methods:** This cross-sectional and descriptive study, which included students registered to Medical Faculty at Kuwait University in the academic year of 2012–2013. Out of 808 registrants, 621 students accepted to participate in the study. Participants who had two or more headaches in the last 3 months were subjected to two preliminary questions and participants with at least one positive response were asked to perform the validated ID-Migraine™ test. The frequency of headache per month and severity of headache by Numeric Rating Scale (NRS) were reported.

**Results:** Migraine was detected in 173 subjects (27.9%) based on the ID-Migraine™ test. The mean age of the migraine students was  $20.17 \pm 2.29$  (16–25 years). Thirty-seven were male (21.4%) and 136 were female (78.6%). Migraine was significantly more frequent in the last 2 grades (35.5% and 44%,  $p < 0.000$ ). The frequency and the severity of headache were significantly increased during the last 2 grades ( $5.55 \pm 1.34$  and  $7.23 \pm 1.27$ ,  $p < 0.000$ ) ( $6.00 \pm 0.76$  and  $6.68 \pm 1.25$ ,  $p < 0.000$ ) respectively. Stress 43 (24.9%), irregular sleep 36 (20.8%), and much reading 32 (18.5%) were the most common triggering factors.

**Conclusion:** There is a high prevalence of migraine among medical students in Kuwait University. The frequency and severity of headache increase with years of educations.

doi:10.1016/j.jns.2013.07.1786

#### Abstract – WCN 2013

No: 1617

Topic: 8 – Headache

#### A randomized doubled blinded trial of treatment with diamino-oxidase (DAO) in patients with migraine and deficit of enzyme's activity

J. Izquierdo<sup>a</sup>, D. Mon<sup>a</sup>, M. Lorente<sup>b</sup>, L. Soler Singla<sup>a</sup>. <sup>a</sup>Hospital General de Catalunya, Sant Cugat del Vallès, Spain; <sup>b</sup>Universitat Internacional de Catalunya, Sant Cugat del Vallès, Spain

**Background:** Histamine has been considered as a chemical mediator of migraine. The degradation is done in two different pathways. One of the enzymes that allow this process is the diamino-oxidase (DAO).

**Objective:** The aim of this study is to identify the prevalence of the deficit in the activity of DAO in patients with migraine, and test the supplementation of this enzyme in a randomized controlled double-blind trial.

**Material and methods:** This was a randomized parallel-group controlled study. After a 1-month run-in, patients with migraine attacks/month between 4 and 14 were randomized 1:1 to placebo or DAO three times a day during one month. Primary outcome measures were diminution of hours of pain, and the use of antimigraine drugs.

**Results:** We studied 137 patients with migraine, and find the deficit of DAO activity ( $< 80$  HDU/ml) in 119 (87%).

One hundred patients were randomized and included in the intention-to-treat analysis. Between run-in and first month of treatment, the mean number of hours of pain decreases in both groups but with significant difference in the final control in the group treated with DAO compared with placebo (6,3 vs 5,1:  $p < 0.03$ ).

The use of the acute antimigraine drug was significantly reduced in the DAO but not in placebo group ( $p > 0.022$ ).

There were no adverse events in either group.

**Conclusions:** Deficit in the activity of DAO is very prevalent in population with migraine.

The supplementation with the enzyme is effective and safe as a preventive therapy for migraine.

doi:10.1016/j.jns.2013.07.1787

#### Abstract – WCN 2013

No: 1621

Topic: 8 – Headache

**Subdural haematoma as a late complication of spontaneous cerebrospinal fluid hypovolemia (SCH) syndrome:**

**Two case reports**

A. Lopez, A. Hernández, C. Valencia, B. Miguel, N. Giraldo, S. Carrasco, M. Deal Real. *Neurology, General Hospital of Ciudad Real, Ciudad Real, Spain*

**Background:** SCH syndrome is relatively common, and a CSF leakage can be occasionally demonstrated. Subdural haematoma (SDH) has been reported in patients with SCH, mainly in older men, or these displaying longer time to diagnosis of SCH.

**Objectives:** We report two cases that developed SDH after apparent resolution of SCH.

**Material and methods:** A 41 year old man developed severe orthostatic headache and neck stiffness after repeated sneezing. Lumbar puncture showed an opening pressure of 0 mm H<sub>2</sub>O. Cisternography demonstrated cervicothoracic CSF leak. A 43 year old man presented sudden headache after sport activity, highly suggestive of SCH, developing unilateral abducens palsy after several days. Cranial tomography (CT) was normal in both cases, with resolution of symptoms after 3 months of conservative therapy.

**Results:** Three months after the onset, when patients were almost asymptomatic, a control MRI showed subacute bilateral SDH. In both, resolution of the haematomas was verified after some weeks without need of drainage.

**Conclusion:** SCH is characterized by orthostatic headache, low CSF pressure, and sometimes typical MRI image. The development of SDH, although rare, has been reported. In our cases the late development of SDH is remarkable, perhaps related to the persistence of SCH for a long time. We emphasize the importance of monitoring patients with SCH, and consider conservative measures only in cases with a brief course. Epidural patching or surgical repair may prevent potentially serious complications such as SDH. Moreover, SCH should be excluded as a cause of SDH in young patients without risk factors.

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#### Abstract – WCN 2013

No: 1298

Topic: 8 – Headache

**Analysis of MAP0004 subjects with menstrually related migraine vs. non-menstrually related migraine**

S.K. Aurora<sup>a</sup>, B. Lu<sup>b</sup>, E. Connors<sup>b</sup>, X. Li<sup>b</sup>, D. Kellerman<sup>b</sup>, S. Kori<sup>b</sup>.  
<sup>a</sup>Department of Neurology, Stanford University, Stanford, USA;

<sup>b</sup>MAP Pharmaceuticals, Inc., a Wholly Owned Subsidiary of Allergan, Inc., Mountain View, CA, USA

**Background:** Menstrually related migraine (MRM), as defined by The International Classification of Headache Disorders, 2nd edition, occurs from days –2 to +3 of menstruation in <sup>3</sup>2 of 3 menstrual cycles and at other times of the cycle. MRM generally lasts longer and is more severe and difficult to treat than non-MRM. MAP0004 is an investigational product that delivers dihydroergotamine through the lungs via a breath-synchronized metered-dose inhaler.

**Objective:** This post hoc analysis of phase 3 data evaluated the efficacy and tolerability of MAP0004 in MRM vs non-MRM.

**Patients and methods:** This analysis included 149 women from a modified intent-to-treat population who treated MRM (n = 45) and non-MRM (n = 104) with MAP0004. The study used the following clinical end points: pain relief and pain free at 2 h and sustained pain relief and sustained pain free at 2–24 h and 2–48 h.

**Results:** The efficacy of MAP0004 did not differ significantly in MRM vs non-MRM at 2 h (pain relief 62% vs 64%; pain free 36% vs 29%), at 2–24 h (pain relief 53% vs 49%; pain free 31% vs 23%), and at 2–48 h (pain relief 38% vs 38%; pain free 24% vs 15%). No significant differences were found in frequency of adverse events, and no drug-related serious adverse events were reported.

**Conclusion:** In this post hoc analysis of phase 3 data, MAP0004 was similarly effective and well tolerated in treating both MRM and non-MRM.

Study supported by MAP Pharmaceuticals, Inc., a wholly owned subsidiary of Allergan, Inc.

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#### Abstract – WCN 2013

No: 1295

Topic: 8 – Headache

**Analysis of the development of allodynia: Correlation between migraine duration and severity**

B. Lu, X. Li, E. Connors, S. Kori. MAP Pharmaceuticals, Inc., a Wholly Owned Subsidiary of Allergan, Inc., Mountain View, CA, USA

**Background:** Allodynia, the perception of pain from non-nociceptive stimuli, is a clinical presentation of central sensitization. Allodynia is reportedly common during migraine attacks. Although factors leading to development of allodynia are not well understood, duration and severity of migraine have been implicated.

**Objective:** This retrospective analysis evaluated the relationship between allodynia and the duration and severity of migraine to better understand the mechanisms related to migraine-induced central sensitization.

**Patients and methods:** This analysis included 792 patients from the double-blind period of a phase 3, placebo-controlled, randomized clinical trial of an investigational acute treatment for migraine (MAP0004). Baseline pain levels were recorded by patients using an electronic diary, and baseline allodynia data were obtained using a standard questionnaire. Correlations between percentage of patients reporting allodynia, severity of migraine, and duration of migraine were analyzed by Fisher's exact test or Chi-square test, as indicated.

**Results:** At baseline, 53% of patients reported allodynia. The presence of allodynia did not change in relation to the duration of the migraine (Chi-square  $P = 0.2182$ ), regardless of migraine severity (moderate pain, Chi-square  $P = 0.1807$ ; severe pain, Chi-square  $P = 0.5830$ ). Patients reporting severe pain experienced significantly more allodynia (58.4%) than patients with moderate pain (48.2%; Fisher's exact test  $P = 0.0053$ ).

**Diamine Oxidase as a triggering factor for migraine.** J. Izquierdo, Ll. Soler, E. Balaguer, D. Mon, Neurology Service. Capió Hospital General de Catalunya.  
**2012** - 16th Annual Meeting of the Catalan Society of Neurology. 26th Neurology Update Course. 40th Yearly gathering of the Catalan Society of Neurology.

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# 16<sup>th</sup> Annual Meeting of the Catalan Society of Neurology

## 26<sup>th</sup> Neurology Update Course

### 40<sup>th</sup> Yearly gathering of the Catalan Society of Neurology (I)

Vic, Barcelona (Spain), march 15-16 2012

1.

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Diamine Oxidase as a triggering factor for migraine

J. Izquierdo, Ll. Soler, E. Balaguer, D. Mon

Neurology Service. Capio Hospital General de Catalunya.

*Introduction:* Migraine is considered one of the most frequent secondary headaches. There are different ethiopathogenic theories, including the alimentary one. Histamine has a high presence in diet and the capacity to degrade it is different in each subject. It is determined by an enzyme called diamine oxidase (DAO). Our purpose is to measure the activity of this enzyme in patients suffering migraine.

*Patients and methods:* Those patients meeting the International Headache Society criteria for the diagnosis of migraine are included. Patients range from 18 to 65 years old, and they should give informed consent to take blood samples. Deficient activity of DAO is considered to be under 80 HDU/mL.

*Results:* From July to October 2011, 40 patients have been recruited, of which 38 (80%) were women. 38 of them (95%) had decreased DAO activity. The average activity was  $57.41 \pm 7.86$  HDU/mL. The average age was  $39.06 \pm 10.82$  years old.





*Conclusions:* There is a high prevalence of DAO deficiency in migraneous patients. Diamine oxidase supplementation may open new avenues for research in the treatment of migraine.

## **2. Diamine Oxidase in Gastroenterology, Respiratory and Pediatrics**

**Diamine Oxidase Activity Deficit and Idiopathic Rhinitis: A New Subgroup of Non-Allergic Rhinitis?**  
Mayo-Yáñez M, Díaz-Díaz A, Calvo-Henríquez C, Lechien JR, Vaira LA, Figueroa A. **2023**. Life (Basel).

## Article

# Diamine Oxidase Activity Deficit and Idiopathic Rhinitis: A New Subgroup of Non-Allergic Rhinitis?

Miguel Mayo-Yáñez <sup>1,2,\*</sup>, Andrea Díaz-Díaz <sup>3</sup>, Christian Calvo-Henríquez <sup>2,4</sup>, Jerome R. Lechien <sup>5,6,7,8</sup>,  
Luigi A. Vaira <sup>9,10</sup> and Angélica Figueroa <sup>3</sup>

- <sup>1</sup> Otorhinolaryngology—Head and Neck Surgery Department, Complejo Hospitalario Universitario A Coruña (CHUAC), 15006 A Coruña, Spain
  - <sup>2</sup> Clinical Research in Medicine, International Center for Doctorate and Advanced Studies (CIEDUS), Universidade de Santiago de Compostela (USC), 15782 Santiago de Compostela, Spain
  - <sup>3</sup> Epithelial Plasticity and Metastasis Group, Instituto de Investigación Biomédica de A Coruña (INIBIC), Complejo Hospitalario Universitario de A Coruña (CHUAC), Universidade da Coruña (UDC), 15006 A Coruña, Spain
  - <sup>4</sup> Otorhinolaryngology—Head and Neck Surgery Department, Complejo Hospitalario Universitario Santiago de Compostela (CHUS), 15706 Santiago de Compostela, Spain
  - <sup>5</sup> Division of Laryngology & Broncho-Esophagology, EpiCURA Hospital, University of Mons, 7331 Baudour, Belgium
  - <sup>6</sup> Department of Otolaryngology-Head & Neck Surgery, Foch Hospital, School of Medicine, UFR Simone Veil, Université Versailles Saint-Quentin-en-Yvelines (Paris Saclay University), 91190 Paris, France
  - <sup>7</sup> Department of Otolaryngology, Elsan Hospital Poitiers, 86000 Poitiers, France
  - <sup>8</sup> Department of Human Anatomy and Experimental Oncology, Faculty of Medicine, UMONS Research Institute for Health Sciences and Technology, University of Mons (UMons), 7000 Mons, Belgium
  - <sup>9</sup> Maxillofacial Surgery Operative Unit, Department of Medicine, Surgery and Pharmacy, University of Sassari, 07100 Sassari, Italy
  - <sup>10</sup> Department of Biomedical Science, PhD School of Biomedical Science, University of Sassari, 07100 Sassari, Italy
- \* Correspondence: miguel.mayo.yanez@sergas.es



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**Abstract:** Idiopathic rhinitis represents more than 50% of non-allergic rhinitis, a heterogeneous group that involves the symptomatic inflammation of the nasal mucosa. The TRPV1 receptor of unmyelinated C-type neurons appears to be involved in its pathophysiology. Histamine, whose main catabolic enzyme is DAO, is one of the mediators that can activate this receptor. The failure of DAO causes an increase in the level of histamine in the body and, consequently, the activation of TRPV1. The objective was to investigate the existence of a DAO enzyme activity deficit in idiopathic rhinitis and its correlation with symptoms. A cross-sectional study was conducted in 116 idiopathic rhinitis patients, and DAO activity, nasal peak inspiratory flow, and rhinitis severity were recorded. The prevalence of a DAO activity deficit was 41.38% (95%CI 0.33–0.50;  $p = 0.05$ ). The DAO activity in patients with mild rhinitis was  $52.93 \pm 8.72$  HDU/mL, in those with moderate rhinitis it was  $120.33 \pm 71.63$  HDU/mL, and in those with severe rhinitis it was  $92.58 \pm 27.75$  HDU/mL ( $p = 0.006$ ). The NPIF in patients with a DAO activity deficit was  $107.92 \pm 34.05$  L/min, compared to  $72.35 \pm 27.16$  L/min in patients with normal enzymatic activity ( $p < 0.001$ ), demonstrating a linear correlation between activity levels and nasal obstruction ( $-0.45$ ;  $p < 0.001$ ). Therefore, patients with a DAO deficiency and idiopathic rhinitis could present a milder disease course, because the repeated and continuous activation of TRPV1 led to a partial or total decrease in their response (desensitization). This new theory represents a different perspective for the study of idiopathic rhinitis and its relationship with TRPV1, with the regulation or modulation of the desensitization of TRPV1 being an important therapeutic target for patients with idiopathic rhinitis in the future.

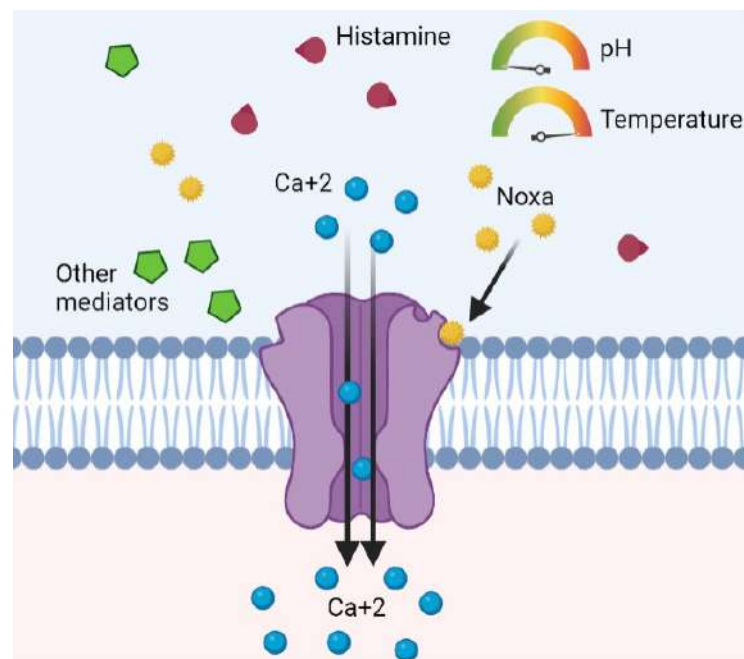
**Keywords:** idiopathic rhinitis; diamine oxidase; histamine; TRPV1; peak nasal inspiratory flow; rhinology; maxillofacial surgery; otorhinolaryngology

## 1. Introduction

Rhinitis is defined as a symptomatic inflammation of the inner lining of the nose, classically divided into allergic rhinitis (AR) and non-allergic rhinitis (NAR). NAR comprises a large heterogeneous group of patients without systemic signs of allergic inflammation (allergen-specific IgE in the blood and/or positive SPT results) or clinical signs of infection. Of these cases, up to 50% do not have a clear etiology underlying the symptoms and are defined as idiopathic rhinitis (IR) [1–3].

Several mechanisms have been postulated to explain IR pathophysiology. One of the most widely accepted suggestions is the nociceptive transient receptor potential vanilloid 1 (TRPV1) signaling pathway, which is found upregulated in IR patients [4,5]. Within the sensory nerve fibers present in organs, TRPV1 is implicated in the detection and integration of thermal and chemical nociceptive stimuli [6]. Moreover, TRPV1 is particularly widely distributed in the sensory fibers of the upper and lower respiratory tracts [7–11].

This receptor can be activated, directly or indirectly, by a large number of mediators: ethanol, tissue damage, mechanical stimuli, acid pH, temperature above 43 °C, osmotic pressure alterations, cations, leukotrienes, nitric oxide, lipoxygenases, capsaicin, substance P, nerve growth factors, and prostaglandins (Figure 1) [6,7,12–17]. Curiously, another mediator capable of activating TRPV1 is histamine, which is the most studied mediator of AR [16].



**Figure 1.** TRPV1 receptor of unmyelinated sensory C fibers is activated by several physiological stimuli, exogenous noxa, or endogenous inflammatory mediators.

Histamine intolerance (HIT), an imbalance between the supply of histamine and the body's ability to degrade it, belongs to the group of pharmacological non-IgE-mediated food intolerances. Its prevalence in the general population is approximately 1%, but this figure is probably underestimated due to the condition's wide spectrum of symptoms [18–21]. Ingested exogenous histamine is distributed in the blood stream and may trigger a wide range of symptoms in the susceptible population. Its main cause is a deficit in histamine degradation due to a genetic, pathological, or pharmacological malfunction of the diamine oxidase (DAO) enzyme, which can cause an increase in the plasma concentration of histamine. The DAO enzyme represents the main enzyme involved in histamine metabolism [18–21], and its activity can be influenced by numerous factors. DAO gene transcription or enzyme function may be reduced in certain gene polymorphisms [20,22,23].

Recent clinical studies have established a relationship between DAO malfunction and specific pathologies, mainly dermatological, respiratory (including AR), gastrointestinal, and neurological [20,22–30]. However, no studies have addressed the relationship between a DAO enzymatic activity deficit and IR and its correlation with symptoms.

The physiology of the TRPV1 receptor and its response to histamine could allow for a subgroup of patients with IR and a DAO activity deficit in which the symptomatology and clinical course vary; therefore, these patients could benefit from personalized treatment. The objective of this preliminary study was to investigate the existence of a DAO enzyme activity deficit in patients diagnosed with IR and its correlation with symptoms.

## 2. Materials and Methods

A cross-sectional study involving patients diagnosed with IR according to the European Academy of Allergy and Clinical Immunology criteria [1] was performed in an otorhinolaryngology head and neck surgery department of a tertiary university hospital.

Candidate patients were required to present at least two of the following symptoms for at least 1 h daily for 12 weeks per year: nasal obstruction, rhinorrhea (anterior or posterior), sneezing, and nasal/ocular itch. Patients with rhinosinusitis and occasional or nasal symptoms were excluded. Selection was based on a structured interview and physical examination (anterior rhinoscopy and flexible fibronasoscopy) revealing no evidence of important nasoseptal deformities or polyps, performed independently by at least one otorhinolaryngologist and one allergist. For each participant, a detailed history of allergies was collected, including a skin-prick test for common aeroallergens. The relevant subgroups of NAR in clinical practice (rhinitis of the elderly, drug-induced rhinitis, hormonal rhinitis including nonallergic occupational rhinitis, pregnancy-induced rhinitis, and gustatory rhinitis) were excluded based on this clinical history, with support from the necessary complementary tests as appropriate. The key feature of IR is the presence of nasal hyperresponsiveness [1]. Since there are no validated methods for the evaluation of nasal hyperresponsiveness that can distinguish its etiology [31], the diagnosis of IR was carried out per exclusionem, on the basis of the symptoms reported by the patients and the absence of a clinical history that could lead us to suspect another possible etiology.

Several diagnostic strategies involving various tests have been proposed, because direct HIT-specific diagnostic criteria are lacking. Following the recommendations of the clinical guidelines, only cases matching the analytical criteria and presenting a compatible clinical history (history of flushing, itching, diarrhea, nausea/vomiting, abdominal pain, dyspnea, rhinitis, dysphonia, dizziness, low blood pressure, or tachycardia) were classified as DAO deficiency [20,21,32–34]. It was necessary to analyze the symptoms with reference to their temporal onset for the differential diagnosis, because adverse food reactions are only suspected in the case of a temporal relationship (min <4 h) to food intake.

Only adults over 18 years old were included. The exclusion criteria were: pregnancy; treatment with the DAO enzyme for the past 2 weeks; treatment with any medication that may have caused a secondary decrease (antihistamines, aminophylline, systemic corticosteroids, cefuroxime, verapamil, metoclopramide clavulanic acid, etc.) or increase (heparin) in DAO activity and could not be suspended [18,19,21]; a history of histamine-rich aliment intake in the past 2 weeks (fermented foods, beverages, processed meat or seafood); and medical disorders (severe hepatic, renal, or gastrointestinal disease; zinc, copper, and vitamin B6 or C deficiency, etc.) that could alter the analytical results.

### 2.1. Data Collection

Sociodemographic variables; DAO enzyme activity; nasal peak inspiratory flow; and IR severity score (mild 0–3, moderate 4–7, and severe 8–10) based on a visual analogue scale (VAS, 0–10 cm calibrated) were recorded [34,35].

Nasal peak inspiratory flow (NPIF) is a rapid and simple technique performed using a plastic cylinder calibrated between 30 and 370 L/min with a facial mask attached. In this study, we used the In-Check portable inspiratory flow meter (Clement Clarke International

Ltd., Harlow, UK). From an expiratory maneuver to residual volume, a forced inspiration was made while the lips were sealed. The best of three measurements that varied by no more than a 10% was chosen. Normal reference values for Caucasian adults are  $143 \pm 48.6$  L/min for males and  $121.9 \pm 36$  L/min for females [35,36].

DAO enzyme activity was determined by ELISA in accordance with the manufacturer's instructions (D-HIT, Sciotec, Donau, Austria) and previous literature reports, with a venipuncture after an 8 h fasting period in an EDTA tube [37]. The threshold for serum DAO enzyme activity has been proposed to be 80 HDU/mL, with a value below this figure indicative of a DAO activity deficiency; in such a case, the presence of HIT should be considered. One HDU corresponds to the DAO activity needed to degrade 1 pmol/mL (0.11 ng/mL) of histamine. The usefulness of DAO measurement in HIT as a marker of disease and disease severity and as a predictor of treatment response has been validated in several studies [38,39].

In order to exclude patients with allergies, a skin-prick test with a calibrated lancet (1 mm) held vertically while introducing a drop of diluted purified allergen was used. According to the protocol of the Hospital's Allergology Service, and based on the allergens typical of the area in which the study was carried out, the extracts included were *Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Gliciphagus*, *Blomia tropicalis*, *Chortoglyphus*, *Alternaria alternata*, *Phleum pratense*, *Cynodon dactylon*, *Plantago lanceolata*, *Parietaria judaica*, *Betula verrucosa*, *Populus nigra*, *Cupressus arizonica*, *Platanus acerifolia*, latex, profilina, and dog and cat epithelium. A drop of histamine (10 mg/mL) and saline solution were used as a positive and negative control, respectively. The maximum or mean diameters of the wheals for various allergens were read at 15 min. A wheal  $\geq 2$  mm in diameter was considered positive, indicating sensitization to the allergen.

## 2.2. Statistical Analysis

Statistical analysis was performed with R program 3.6.1 (The R Foundation for Statistical Computing<sup>®</sup>, Vienna, Austria). Normality was evaluated by the Kolmogorov–Smirnov test and variances using the Levene test. All tests were two-tailed with a 95% confidence interval (CI). Quantitative variables were expressed as mean  $\pm$  standard deviation (SD) and/or median, as appropriate. For the qualitative variables, frequency and percentage were used. The comparison of means was performed using Student's *t* test, while qualitative or categorical differences between groups were evaluated by the  $\chi^2$  test or Fisher's exact test, as appropriate. In cases where non-normality was significant, non-parametric methods were applied. Correlation between variables was assessed using Pearson's test or Spearman's test, as appropriate.

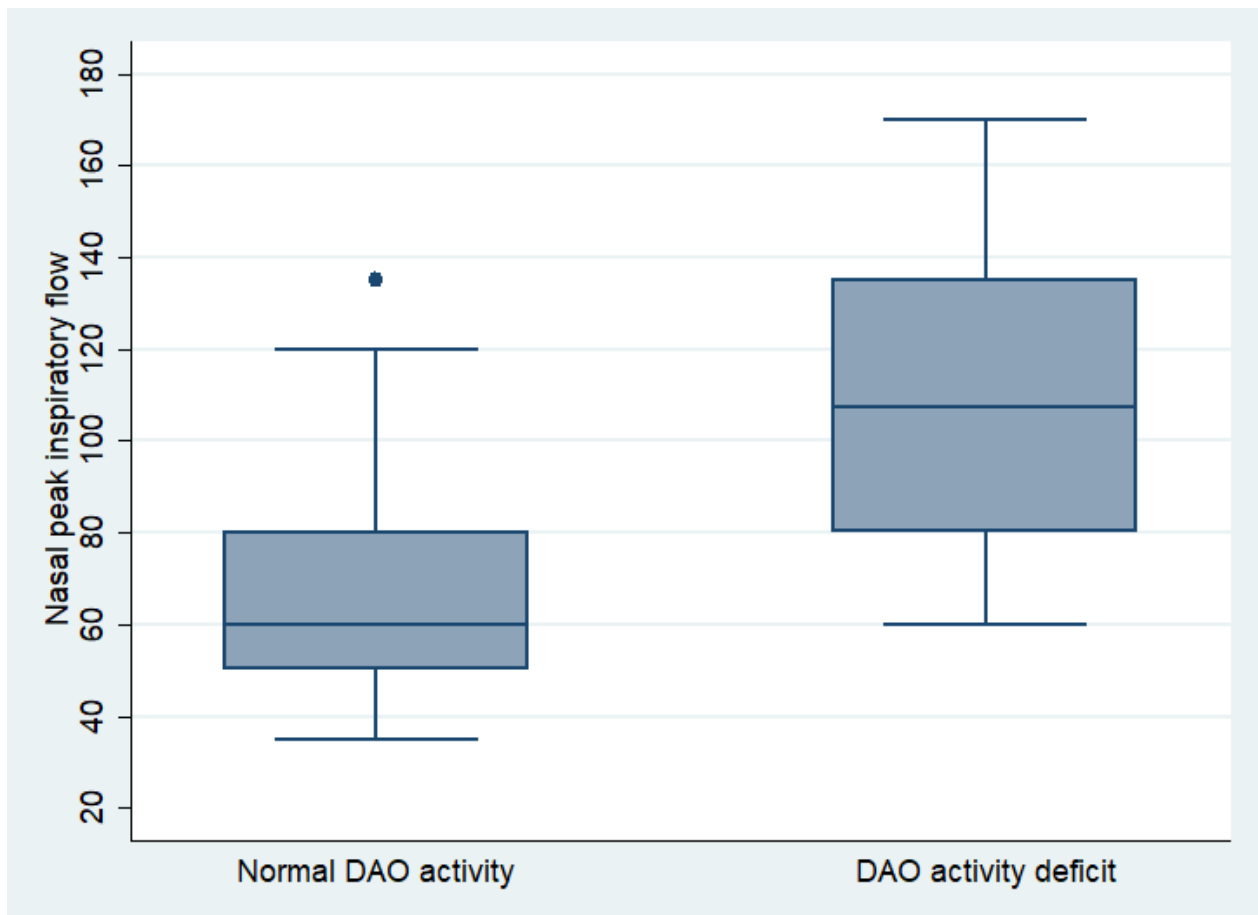
## 3. Results

A total of 116 Caucasian patients with IR were recruited: 20 men (17.24%) and 96 (82.76%) women. The mean age was  $45.36 \pm 11.41$  years (men:  $35.61 \pm 15.96$  years; women:  $47.39 \pm 9.08$  years). The prevalence of DAO activity deficits in patients with IR was 41.38% (95%CI, 0.33–0.50). The mean DAO enzyme activity of the 116 patients was  $108.98 \pm 63.9$  HDU/mL:  $85.60 \pm 43.15$  HDU/mL in men and  $113.85 \pm 66.57$  HDU/mL in women ( $p = 0.161$ ). The mean DAO activity in the normal-function group (i.e., patients with DAO activity  $\geq 80$  HDU/mL) was  $143.05 \pm 63.96$  HDU/mL, compared to  $60.72 \pm 10.16$  HDU/mL in the group with an activity deficit ( $p < 0.000$ ).

According to the severity of the symptoms measured using the VAS, 8 (6.90%) patients had "mild" rhinitis, 80 (68.97%) "moderate" rhinitis, and 28 (24.14%) "severe" rhinitis. The global mean was  $6.35 \pm 1.78$  points, being  $5.6 \pm 2.3$  for men and  $6.51 \pm 1.63$  for women ( $p = 0.393$ ). After stratification according to severity groups, the mean score was 2 in the mild IR group,  $6.05 \pm 0.89$  in the moderate IR group, and  $8.50 \pm 0.73$  in the severe IR group ( $p < 0.001$ ). The DAO activity in patients with mild rhinitis was  $52.93 \pm 8.72$  HDU/mL, in

moderate rhinitis it was  $120.33 \pm 71.63$  HDU/mL, and in severe IR it was  $92.58 \pm 27.75$  HDU/mL ( $p = 0.006$ ).

The mean NPIF of all patients was  $87.06 \pm 34.82$  L/min, being  $89 \pm 26.23$  L/min for men and  $86.67 \pm 36.46$  L/min for women ( $p = 0.514$ ). In the mild group, the NPIF was  $85 \pm 10.69$  L/min, in the moderate group it was  $88.25 \pm 37.29$  L/min, and in the severe group it was  $84.28 \pm 32.48$  L/min ( $p = 0.904$ ). The NPIF in patients with a DAO activity deficit was  $107.92 \pm 34.05$  L/min, compared to  $72.35 \pm 27.16$  L/min in patients with normal enzymatic activity ( $p < 0.001$ ) (Figure 2), demonstrating a linear correlation between activity levels and nasal obstruction ( $r = -0.45$ ;  $p < 0.001$ ).



**Figure 2.** Comparison of nasal peak inspiratory flow between patients with normal DAO activity and DAO activity deficit.

#### 4. Discussion

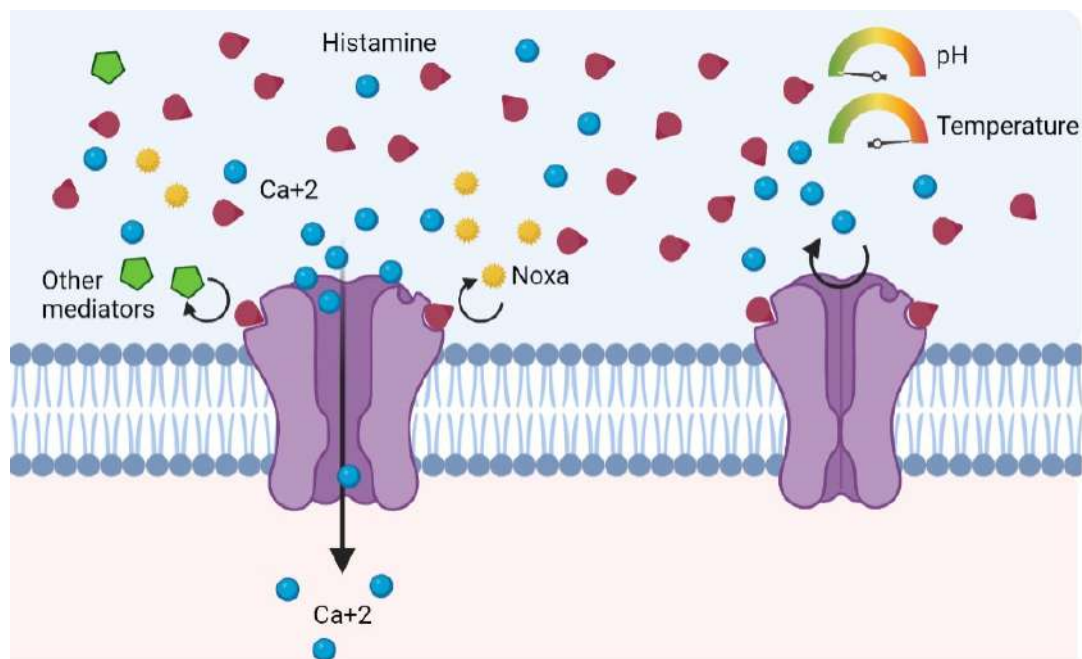
HIT, also known as sensitivity to dietary histamine or enteral histaminosis, can be defined as a dysfunction caused by a reduction in DAO activity that affects the degradation of histamine in the intestine. Under physiological conditions, DAO creates an intestinal enzymatic barrier that protects the body from the absorption of histamine ingested with food [19,20]. HIT provokes a wide range of symptoms (extraintestinal and gastrointestinal) due to the fact that histamine receptors are widespread in all tissues of the body. This amount of histamine is usually well-tolerated in healthy individuals [18,19].

IR represents a diagnostic and therapeutic challenge [1]. The mechanisms involved in its pathophysiology are still unknown, but the TRPV1 receptors have become more relevant, since the activation of this ion channel causes the release of the calcitonin G-related and substance P neuropeptides from nerve terminals, causing a local inflammatory reaction. Several publications have established a connection between TRPV1 activation in the air-

ways and asthma or NAR [6,7]. The activation of the unmyelinated C-fibers where these receptors are present provokes bronchoconstriction; mucus secretion; bradycardia; itching; pain; the irritation of the airways; vasodilation; edema; and, due to the chemotactism, the recruitment, differentiation, and activation of many immune cells (eosinophils, lymphocytes, macrophages, and mast cells). This inflammatory reaction is called “neurogenic inflammation” [40].

The activity and efficiency of TRPV1 under physiological conditions are low. An inflammatory process activates peripheral sensory nociceptors that are capable of locally releasing numerous pro-algesic chemical mediators, such as histamines, serotonin, bradykinins, prostaglandins, cytokines, and growth factors. All of these contribute to enhancing the local inflammation process, and consequently, the function of TRPV1 is synergistically enhanced by the simultaneous action of these released pro-inflammatory substances. These substances cause an increase in the sensitivity and response of peripheral nociceptors, which leads to a decrease in the activation threshold of these terminals and an increase in the magnitude of their response. This phenomenon is known as peripheral sensitization [41,42].

One particularity of TRPV1 is that the repeated and continuous activation of nociceptors leads to a partial or total decrease in their response. The TRPV1 becomes refractory, being unable to respond to new stimuli (Figure 3) [10,43]. This state is known as desensitization [44,45]. Likewise, prolonged exposure to agonists, such as histamine in this case, promotes the endocytosis of the receptor and subsequent lysosomal degradation, perpetuating the state of desensitization and decreased response to stimuli [46]. One possibility is that the increase in circulating histamine due to HIT causes an increase in vasodilation and chemotactism locally in the nasal mucosa, facilitating the release of histamine from mast cells. This in turn would activate or sensitize primary afferent C-fibers via TRPV1 [13,47].



**Figure 3.** Continuous activation and consequent refractory (desensitization) due to histamine action as a competitive agonist.

Histamine could act as a competitive agonist against other molecules, displacing a possible stronger response to the activation of TRPV1. In fact, previous studies suggest that the increase in calcium concentrations stimulated by histamine is slower, with a lower amplitude and shorter duration compared to capsaicin activation, even in the continuing presence of the agonist [48]. Furthermore, a prolonged state of desensitization could induce

the endocytosis of the TRPV1 receptors, causing a decrease in the response to stimuli [10]. The repeated stimulation of TRPV1 by a non-physiological increase in histamine due to a deficit in its degradation could cause this phenomenon. Future studies are needed to clearly elucidate the potential implications of this hypothesis. In fact, contrary to what happens in acute desensitization, the pathways involved in the medium-to-long-term desensitization of the receptor have been barely studied [10].

In this study, DAO activity was investigated in patients diagnosed with IR. Other authors have studied serum or intracellular DAO concentrations [27]. Nevertheless, the present study was the first to use DAO activity and not only its concentration, thus succeeding in exploring the relationships between IR symptoms and alterations in histamine metabolism related to a DAO activity deficiency. The prevalence of DAO deficiencies found in our study was consistent with the results of other studies addressing different pathological conditions such as chronic urticaria, atopic eczema, chronic abdominal pain, and lactose malabsorption, which reported values ranging between 8% and 57% [20,22–25]. Another important aspect for future studies is the concurrent investigation of rhinitis and laryngopharyngeal reflux [49]. Indeed, some recent data suggested a potential relationship between HIT and laryngopharyngeal reflux [50], the latter possibly being involved in rhinitis.

Several authors have detected a correlation between DAO concentrations and female sex hormone changes [18], but this was not observed in our case series for DAO activity. Approximately 40% of the IR patients with a clinical history and analytical results compatible with a DAO activity deficit presented better NPIF results compared to the normal-DAO-activity group. This finding suggests the existence of a subgroup of patients diagnosed with IR whose clinical presentation differs from the rest. Past studies have assessed DAO activity, and three non-synonymous single-nucleotide polymorphisms (SNPs) have been mapped to the DAO gene (chromosome 7q34–36). Functional impairment was related to serum DAO activity only for the SNP with the refSNP ID rs1049793 (NCBI SNP database), which codes for an altered protein with the amino acid substitution His645Asp [23]. Similar studies analyzing the prevalence of DAO deficiency in AR have demonstrated the existence of this mutation in 38% of AR patients, a figure very similar to the prevalence of enzymatic impairment found in our study [3,22]. Contrary to the findings in AR patients, in whom serum DAO was positively correlated with severity [24,27,28,30], our work showed a moderate inverse correlation. This was in line with the hypothesis previously stated. This difference in AR and IR symptomatology according to DAO activity seems to be linked to the mechanism of histamine activation in each case. While the inflammatory pathway in IR would be via TRPV1, in the case of AR, it is an IgE-mediated response with Th2 activation [5].

An important limitation of this study is the impossibility of extrapolating the prevalence of DAO activity deficiencies in the general population, as the patients were recruited only from the outpatient department, and there was no control group. For this reason, it is not possible to conclude whether the IR subgroups established on the basis of DAO activity represent the variability of the general population or non-IR patients. Patients' motivation to seek care can be influenced by symptom severity, introducing an inclusion bias. In other words, patients with very mild or very severe symptoms may not recognize them as pathological [3,51]. This may have influenced the severity distribution of IR found in the sample. Other limitations include the lack of other parameters (serum histamine, IgE) and the absence of a prospective evaluation with repeated measures of DAO activity over time [52,53]. Finally, only 17% of the patients included in the study were men, and it is possible that this introduced selection bias, affecting the results. The strengths of this study include the physiological basis previously demonstrated in the literature, on which the hypothesis was based; the evaluation of the NPIF; and the first ever investigation of the relationship between histamine intolerance and DAO activity in IR patients [31,33,36,54,55].

Although our results were not sufficient to definitively clarify the link between IR severity and DAO activity, this study opens up a new range of possibilities. No study has

addressed the role of DAO SNPs in patients with IR. It can be hypothesized that, as in the case of AR, the presence of these polymorphisms may modify the pathogenesis of IR due to the involvement of DAO in the metabolism of circulating histamine.

## 5. Conclusions

The hypothesis of this manuscript was that the repeated stimulation of TRPV1 could be caused by supra-physiological histamine levels generated due to a deficit in their degradation, producing a less severe symptomatology in a subgroup of patients with IR. The results of the pilot study supported this claim: patients with mild rhinitis had lower DAO activity than patients with moderate and severe rhinitis, and the decrease in DAO activity was inversely proportional to nasal obstruction assessed according to NPIF. This represents a new perspective for the study of IR and its relationship with TRPV1, with the regulation or modulation of the desensitization of TRPV1 being an important therapeutic target for patients with IR in the future.

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## EDITED BY

Balamurugan Ramadass,  
All India Institute of Medical Sciences,  
Bhubaneswar, India

## REVIEWED BY

Mario Di Gioacchino,  
University of Studies G. d'Annunzio  
Chieti and Pescara, Italy  
Brantley Hall,  
University of Maryland, College Park,  
United States

## \*CORRESPONDENCE

Oriol Comas-Basté  
oriolcomas@ub.edu  
M. Teresa Veciana-Nogués  
veciana@ub.edu  
M. Carmen Vidal-Carou  
mcvidal@ub.edu  
M. Luz Latorre-Moratalla  
mariluzlatorre@ub.edu

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# The dietary treatment of histamine intolerance reduces the abundance of some histamine-secreting bacteria of the gut microbiota in histamine intolerant women. A pilot study

Sònia Sánchez-Pérez<sup>1,2,3</sup>, Oriol Comas-Basté<sup>1,2,3\*</sup>,  
Adriana Duelo<sup>1,2,3</sup>, M. Teresa Veciana-Nogués<sup>1,2,3\*</sup>,  
Mercedes Berlanga<sup>4</sup>, M. Carmen Vidal-Carou<sup>1,2,3\*</sup> and  
M. Luz Latorre-Moratalla<sup>1,2,3\*</sup>

<sup>1</sup>Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Campus de l'Alimentació de Torribera, Universitat de Barcelona (UB), Santa Coloma de Gramenet, Spain, <sup>2</sup>Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona (UB), Santa Coloma de Gramenet, Spain, <sup>3</sup>Xarxa d'Innovació Alimentària (XIA), Barcelona, Spain, <sup>4</sup>Departament de Biologia, Sanitat i Mediambient, Secció de Microbiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Barcelona, Spain

Restrictive diets for the treatment of different gastrointestinal disorders are reported to change the composition of intestinal microbiota. Recently, it has been proposed that individuals with histamine intolerance suffer from intestinal dysbiosis, having an overabundance of histamine-secreting bacteria, but how it is still unknown this state is affected by the usual dietary treatment of histamine intolerance [i.e., low-histamine diet and the supplementation with diamine oxidase (DAO) enzyme]. Thus, a preliminary study was carried out aiming to evaluate the potential changes on the composition of the intestinal microbiota in a group of five women diagnosed with histamine intolerance undergoing 9 months of the dietary treatment of histamine intolerance. After sequencing bacterial 16S rRNA genes (V3-V4 region) and analyzing the data using the EzBioCloud Database, we observed a reduction in certain histamine-secreting bacteria, including the genera *Proteus* and *Raoultella* and the specie *Proteus mirabilis*. Moreover, it was also observed an increase in *Roseburia* spp., a bacterial group frequently related to gut health. These changes could help to explain the clinical improvement experienced by histamine intolerant women underwent a dietary treatment.

## KEYWORDS

histamine intolerance, intestinal microbiota, gut dysbiosis, low-histamine diet, DAO supplementation, histamine-secreting bacteria