Incidence of a clinically relevant H63D syndrome in carriers of a homozygous mutation of HFE gene H63D

Jacob S. Adams\textsuperscript{IC}, Marianne Kaufmann\textsuperscript{IC}, Riku Honda\textsuperscript{IC}, David Seideman\textsuperscript{LCG}, Carolina Diamandis\textsuperscript{LCG}

Affiliations:

Lazar Clinic Group (LCG)
Rare Diseases Research Consortium
(non-profit)

International H63D Consortium (IC)
(non-profit)

Corresponding Author:

Dr. Carolina Diamandis
LCG Greece
Rare Diseases Research Consortium
Kifissias 16, Athina, 115 26
Hellenic Republic
carolina.diamandis@europe.com

Abstract

H63D syndrome is a phenotype of a homozygous mutation of the HFE gene H63D, which is otherwise known to cause at most mild classical hemochromatosis. H63D syndrome leads to an iron overload in the body (especially in the brain, heart, liver, skin and male gonads) in the form of non-transferrin bound iron (NTBI) poisoning. Hallmark symptoms and causal factor for H63D syndrome is a mild hypotransferrinemia with transferrin saturation values >50%. H63D syndrome is an incurable multi-organ disease, leading to permanent disability. Our objective was to find out how many carriers of a homozygous H63D mutation develop H63D syndrome. For this purpose, we systematically evaluated the medical records of homozygous carriers of the mutation. We found the syndrome in about 10% of patients with a homozygous mutation. Since a homozygous mutation on the HFE gene H63D is relatively common, the results of our study suggest many undetected or misdiagnosed cases.
**Introduction**

Only very rarely a homozygous mutation of the HFE gene H63D can lead to hemochromatosis. Therefore, this mutation is usually wrongly considered clinically not very relevant in comparison to other HFE mutations. However, this is not correct. We reported typical symptom constellations in patients with a homozygous mutation of the HFE gene H63D who have developed H63D syndrome in an earlier study. Unlike what is the case in hemochromatosis, the syndrome does not result from ferritin overload but from accumulation of non-transferrin-bound iron (NTBI) caused by a trigger-unresponsive hypotransferrinemia.\(^2,7,26\)

NTBI has the ability to enter numerous cell types and calcium channels. In the cells, it leads to degeneration processes. In advanced stages, therefore, brain damage (especially in the substantia nigra and basal ganglia), cardiac muscle damage or conduction disorders (e.g. heart blocks) may occur. In the cells, NTBI leads to oxidation processes that damage or destroy the affected cells affected and variable dysfunction of the liver are also among the symptoms found in H63D syndrome. The skin shows hyperresponsiveness, and urologists find mildly atrophic testes in affected men. The brain, heart, liver, skin and, in males the testes are virtually always affected.\(^1-7\)

H63D syndrome is still an incurable multi-organ disease, leading to permanent and often severe disability, which can only be influenced by early diagnosis and a very careful reduction of iron intake (under constant medical monitoring) as early as in childhood and youth. Phlebotomies or dialysis are ineffective in this disease. Bloodletting only causes further loss of vital ferritin. Dialysis does also not lead to a clinically favorable result. The NTBI type iron remains in the cells until they die, only to immediately "move" to a nearby cell. Filtering NTBI from the blood is possible, but due to the described behavior of NTBI, the success would be very limited.\(^15,20\)

Another factor makes the procedure of dialysis completely useless in H63D syndrome: the basic pathomechanism of the disease is a non-responsive hypotransferrinemia. Since patients with H63D syndrome also need ferritin for survival, a completely iron-free diet is out of the question. Therefore, the "success" of any filtering of NTBI from the blood would be nullified with the next meal. The fact that some physicians nevertheless recommend phlebotomies or filtration therapies can at best be explained by a lack of knowledge. In any case, it is to be warned against it.\(^2,5-7\)

**Method**

We had anonymized access to the patient records of 147 (65 men, 82 women aged 18 to 72 years) patients with confirmed HFE gene H63D mutation through the H63D Syndrome Research Consortium. We systematically searched these files for the typical constellation of hallmark symptoms of H63D syndrome:
• Neurological symptoms such as tics and/or other movement disorders and/or dementia and/or thought disorders (especially those of obsessive nature, drop in IQ) and/or psychiatric symptoms and/or loss of sense of smell [7 POINTS]
• Conduction disturbances in the heart (heart blocks) and related conditions under the age of 50 [4 POINTS]
• Liver dysfunction [3 POINTS]
• Autoimmune-like reactions, restricted to skin condition [2 POINTS]
• Signs of testicular degeneration in sonography [2 POINTS] $m$ only
• Difficulties in conceiving [2 POINTS] $f$ only

A score was assigned to each of the clinically significant symptom complexes. If a minimum additive score of 11 could be found based on the medical record of a patient with HFE H63D gene mutation, he or she was classified as a "case" with respect to H63D syndrome. Because of this simplistic but clear methodology, the retrospective diagnostic process could be carried out on the basis of the records solely while preserving full anonymity of the patients. Inconclusive findings were rated as negative and no point value was assigned. This restrictive approach may have led to some under-reporting of patients with H63D syndrome.

**Results**

Eight of the 65 male patients and nine of the 82 female patients scored high enough to be considered a H63D syndrome case. The rate was higher in older patients than in younger ones, consistent with the progressive nature of H63D syndrome. In this respect, this factor may also have led to an underreporting of cases.

1) **Patients retrospectively diagnosed H63D syndrome cases:**

   | Gender  | Percentage |
   |--------|------------|
   | Males  | ~12.3%     |
   | Females| ~11.0%     |

It was noteworthy that the cases were well above the threshold of 11 points, while the rest were well below:

   Average points non-cases: 5.7
   Average points cases: 14.2

  *The difference is highly significant.*

A gender dependence of the values could not be determined. Also worth mentioning is a detected frequency of low-grade eosinophilia, sometimes also basophilia in H63D syndrome cases. 67% of H63D patients over 45 years of age were considered severely disabled (in each case according to the legal situation in their home country), whereas in the group without H63D syndrome this was the case in only 12%:
2) Status “severely disabled” (granted by local authorities):

| Status                                      | Percentage |
|---------------------------------------------|------------|
| H63D syndrome patients                      | 67%        |
| H63D mutation carriers (homozygous) without the syndrome | 12%        |

The difference is highly significant.

3) H63D syndrome patients wrongly classified as mentally ill

| Gender | Percentage |
|--------|------------|
| Males  | 92%        |
| Female | 96%        |

4) H63D syndrome patients with other wrong diagnoses:

| Gender | Percentage |
|--------|------------|
| Male   | 100%       |
| Female | 100%       |

We thus found cases of H63D syndrome in a good one tenth of the carriers of a homozygous mutation of the HFE H63D gene. The affected persons were informed about the result, if they had requested this when giving their consent to the use of their anonymized data. This was also done anonymously via the possibility of retrieving the result by means of a code with a verification number. It was ensured that the result could only be accessed in the presence of a physician in order not to leave any positive patient alone with his/her result.

Discussion

The reasonable assumption\textsuperscript{26} that the H63D syndrome is more often a clinical consequence of a homozygous mutation at the HFE gene H63D was again confirmed by this retrospective study. Considering the relatively high prevalence of this mutation\textsuperscript{7-9}, the well over 10% of undiagnosed or misdiagnosed patients represent a substantial group of individuals suffering from a mostly severe syndrome. To make matters worse, the course of the disease is strongly influenced by how much NTBI is formed and accumulates in the body. Since neither phlebotomies nor chelation therapies are helpful in persistent NTBI intoxication, the only magic bullet to prevent progression is a finely tuned diet that contains exactly the amount of iron that leads to sufficiently high ferritin levels, but without driving transferrin saturation to levels >50% due to unresponsive hypotransferrinemia. This is the only way to prevent the constant re-formation of NTBI and its storage in the brain and parenchymal cells.\textsuperscript{2, 7, 28, 29}

References to the successful elimination of NTBI from the body refer exclusively to conditions with a one-time massive iron overload in the context of iatrogenic interventions. They are not applicable to patients who produce NTBI day after day themselves.\textsuperscript{19}
Since the symptom burden increases with age, which we have been able to confirm, the diagnosis must be made in the first decade of life if possible, whereupon a medically supervised diet keeps dietary iron intake within a narrow acceptable corridor. However, this approach is hardly feasible for practical reasons. First, it would require genetic testing of every single individual in the first decade of life. Second, it would impose an undue burden if a homozygous mutation at the HFE gene H63D were actually detected. After all, about 90% of "positives" will never develop a clinically relevant H63D syndrome, let alone hemochromatosis. Moreover, to date, no one knows the reason why the penetrance of the mutation is relatively low. Therefore, routine screening is not ethically justifiable.

Thus, the crucial moment to positively influence the fate of a person with H63D syndrome, in such a way that the path to severe disability can be avoided, is the time when the typical constellation of symptoms starts to appear in a clinically relevant way.

For example, an adolescent usually has not at the same time a fatty liver at a normal BMI, heart palpitations, microlithiasis, and a thought disorder of the obsessive-compulsive type, possibly even with tics. However, due to the fragmentation of medicine into more and more specialties, such a patient is still neither properly diagnosed nor properly treated. The hepatologist will prescribe a healthy diet and exercise, the cardiologist will think of cardiac phobia (since here mild functional abnormalities occur long before structural damage), the urologist will advise follow-up checks, and in no time the patient ends up with a behavioral therapist on the grounds of alleged learned anxiety and obsessive thinking. This is not only insane, it is the moment when the patient's fate is decided. A young patient with H63D syndrome does not need a psychologist, but a good gastroenterologist and nutritionist, exactly as in Wilson's disease. If this moment is missed, the road to disaster is set.

Conclusions

In patients with the typical symptom pattern of H63D syndrome, repeated simple but timely (before the age of 21) testing of ferritin, transferrin and transferrin saturation and, if necessary, subsequent genetic testing is sufficient to favorably influence the course of the disease and prevent lifelong suffering with severe disability. Failure to recognize an H63D syndrome is already a serious case of medical malpractice even with the currently still limited state of research. The syndrome’s incidence in homozygous carriers of a mutation in HFE gene H63D is about 10%.

Limitations

This paper has several limiting factors. Thus, no patients with heterozygous mutation of the HFE gene H63D were included, because there is no evidence of H63D syndrome in this group. Furthermore, the diagnosis was made retrospectively, anonymously and based on medical records. Nevertheless, the results are plausible and consistent with the “White Paper” of the International H63D Syndrome Research Consortium.
Acknowledgements

We thank all individuals who were willing to volunteer for this study.

Source of funding and conflicts of interest

No external funding.
No conflicts of interest.

Ethical standards, data safety compliance, patient’s rights, and nature of this scientific work

This article is about the scientific classification of defined medical parameters to identify specific symptom clusters. It is not reporting on a clinical trial (or anything similar), especially not a prospective one. All participating subjects gave informed consent for their inclusion. The study was conducted in accordance with the Declaration of Helsinki. Ethical, data protection, and patient rights requirements of the countries from which data were provided or in which these data were used for research purposes were complied with. The examination results of the participating patients were completely anonymized and transmitted to the study personnel with codes that could not be traced. Thus, at no time were personal data generated that could allow conclusions to be drawn about identities.

Raw data

While this study is in pre-print status, raw data from this study is available upon request.

References

1. Kostas Pantopoulos: Inherited Disorders of Iron Overload. Front. Nutr. 5:103. doi: 10.3389/fnut.2018.00103

2. Wint Nandar, James R. Connor: HFE Gene Variants Affect Iron in the Brain. The Journal of Nutrition, Volume 141, Issue 4, April 2011

3. Dekker MC, Giesbergen PC, Njajou OT, van Swieten JC, Hofman A, 127. Breteler MM, van Duijn CM. Mutations in the hemochromatosis gene (HFE), Parkinson’s disease and parkinsonism. Neurosci Lett. 2003;348:117–119.

4. Steven M. LeVine, James R. Connor, Hyman M. Schipper: Redoxactive Metals in Neurological Disorders. New York Academy of Sciences, 2004.
5. Sareen S. Gropper, Jack L. Smith, Timothy P. Carr: Advanced Nutrition and Human Metabolism. Cengage Learning, 7th edition, Boston 2016.

6. Bartzokis G, Lu PH, Tishler TA, Peters DG, Kosenko A, Barrall KA, Finn JP, Villablanca P, Laub G, Altshuler LL, Geschwind DH, Mintz J, Neely E, Connor JR: Prevalent iron metabolism gene variants associated with increased brain ferritin iron in healthy older men. J Alzheimers Dis. 2010 Apr;20(1):333–341.

7. Brissot P, Ropert M, Le Lan C, Loreal O. Non-transferrin bound iron: a key role in iron overload and iron toxicity. BBA Gen Subjects (2012) 1820:403–10. doi: 10.1016/j.bbagen.2011.07.014

8. Athiyarath R, Arora N, Fuster F, Schwarzenbacher R, Ahmed R, George B, et al. Two novel missense mutations in iron transport protein transferrin causing hypochromic microcytic anaemia and haemosiderosis: molecular characterization and structural implications. Br J Haematol. (2013) 163:404– 7. doi: 10.1111/bjh.12487

9. Akbas N, Hochstrasser H, Deplazes J, Tomiuk J, Bauer P, Walter U, Behnke S, Riess O, Berg D.: Screening for mutations of the HFE gene in Parkinson’s disease patients with hyperechogenicity of the substantia nigra. Neurosci Lett. 2006;407:16–19.

10. Borie C, Gasparini F, Verpillat P, Bonnet AM, Agid Y, Hetet G, Brice A, Durr A, Grandchamp B.: Association study between iron-related genes polymorphisms and Parkinson’s disease. J Neurol. 2002; 249: 801–804.

11. Dekker MC, Giesbergen PC, Njajou OT, van Swieten JC, Hofman A, 127. Breteler MM, van Duijn CM. Mutations in the hemochromatosis gene (HFE), Parkinson’s disease and parkinsonism. Neurosci Lett. 2003;348:117–119.

12. Guerreiro RJ, Bras JM, Santana I, Januario C, Santiago B, 120. Morgadinho AS, Ribeiro MH, Hardy J, Singleton A, et al.: Association of HFE common mutations with Parkinson’s disease, Alzheimer’s disease and mild cognitive impairment in a Portuguese cohort. BMC Neurol. 2006;6:24.

13. Fujii H, Takagaki N, Yoh T, Morita A, Ohkawara T, Yamaguchi K, Minami M, Sawa Y, Okanoue T, Ohkawara Y, Itoh Y: Non-prescription supplement-induced hepatitis with hyperferritinemia and mutation (H63D) in the HFE gene. Hepatol Res. 2008 Mar;38(3):319–323.

14. Castiella, Urreta, Zapata et al.: H63/H63D genotype and the H63D allele are associated in patients with hyperferritinemia to the development of metabolic syndrome. Eur J Intern Med. 2019 Nov 30. doi:10.1016/j.ejim.2019.11.021.
15. Gkouvatsos K, Papanikolaou G, Pantopoulos K. Regulation of iron transport and the role of transferrin. Biochim Biophys Acta (2012) 1820:188–202. doi: 10.1016/j.bbagen.2011.10.013

16. Mitchell RM, Lee SY, Simmons Z, Connor JR: HFE polymorphisms affect cellular glutamate regulation. Neurobiol Aging. 2009.

17. Wint Nandar, James R. Connor: HFE Gene Variants Affect Iron in the Brain. The Journal of Nutrition, Volume 141, Issue 4, April 2011, 729S–739S, doi:10.3945/jn.110.130351

18. Borie C, Gasparini F, Verpillat P, Bonnet AM, Agid Y, Hetet G, Brice A, Durr A, Grandchamp B.: Association study between iron-related genes polymorphisms and Parkinson’s disease. J Neurol. 2002; 249: 801–804.

19. Steven M. LeVine, James R. Connor, Hyman M. Schipper: Redoxactive Metals in Neurological Disorders. New York Academy of Sciences, 2004.

20. Valenti L, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, Canavesi E, Lattuada E, Roviaro G, Marches G, Fargion S.: HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology. 2010 Mar;138(3):905–912.

21. P. Adams, P. Brissot, L. W. Powell: EASL International Consensus Conference on Haemochromatosis. Journal of Hepatology 2000;33:485–504.

22. de Valk, Addicks, Gosriwatana et al.: Non-transferrin-bound iron is present in serum of hereditary haemochromatosis heterozygotes. Eur J Clin Invest. 2000 Mar;30(3):248-51.

23. G. M. Bishop, T. N. Dang, R. Dringen, S. R. Robinson: Accumulation of Non-Transferrin-Bound Iron by Neurons, Astrocytes, and Microglia. In: Neurotoxicity Research. 19, 2011, S. 443–451, doi:10.1007/s12640-010-9195-x.

24. Jakeman A, Thompson T, McHattie J, Lehotay DC: Sensitive method for nontransferrin-bound iron quantification by graphite furnace atomic absorption spectrometry. Clin Biochem. 2001 Feb;34(1):43-7

25. Diamandis C, Adams J, Seideman D, et al.: H63D-Syndrome: A phenotype caused by a homozygous mutation of HFE gene H63D. April 2021.

26. M. Kelley, N. Joshi, Y. Xie, M. Borgaonkar: Iron overload is rare in patients homozygous for the H63D mutation. In: Canadian Journal of Gastroenterology & Hepatology. April 2014, S. 198–202, doi:10.1155/2014/468521, PMID 24729993, PMC 4071918
28. A. Finkenstedt, M. Schranz, N. Baumgartner et al.: HFE Genotypen, Eisenstatus und Überleben. In: Zeitschrift für Gastroenterologie, Vol. 52 – P65, 2014, doi:10.1055/s-0034-1376049.

29. L. Valenti, A. L. Fracanzani, E. Bugianesi, P. Dongiovanni, E. Galmozzi, E. Vanni, E. Canavesi, E. Lattuada, G. Roviaro, G. Marchesini, S. Fargion: HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. In: Gastroenterology. Vol. 138, No 3, March 2010, S. 905–912, doi:10.1053/j.gastro.2009.11.013, PMID 19931264