

Scientific Critique of Iio et al. (2020)

*Evaluating the Evidence Base for GH1 c.573_578del as a Cause of Proportionate Dwarfism in Dogs and Its Application in Commercial Canine Genetic Testing
With a Case Series of Two Asymptomatic Homozygous ("At Risk") Adult Chihuahuas from a 113-Dog Cohort*

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Paper Under Review

Iio A, Maeda S, Yonezawa T, Momoi Y, Motegi T. *Isolated growth hormone deficiency in a Chihuahua with a GH1 mutation*. Journal of Veterinary Diagnostic Investigation. 2020 Sep;32(5):733–736. doi:10.1177/1040638720938671.

Executive Summary

The Iio et al. (2020) paper is a single-case report describing a 6-month-old female Chihuahua with isolated growth hormone (GH) deficiency, who was homozygous for an in-frame 6-bp deletion in exon 5 of GH1 (c.573_578del). On the basis of this one case, a PROVEAN bioinformatics prediction, and no functional or segregation evidence, the variant has been incorporated into commercial canine genetic testing panels — including Embark Veterinary's — and is reported to breeders as a clinically actionable result.

This critique applies the American College of Medical Genetics and Genomics / Association for Molecular Pathology (ACMG/AMP) 2015 variant interpretation framework to the available evidence and concludes that the variant currently meets criteria for reclassification to Likely Benign, primarily on the basis of two independent observations of asymptomatic homozygous adult dogs (ACMG criterion BS2). An empirical case series drawn from a well-phenotyped 113-dog Chihuahua breeding cohort is presented as direct supporting evidence.

Part 1. The Published Evidence (Iio et al., 2020)

Summary of the study

A single 6-month-old female Chihuahua (body weight 880 g) presented to the Veterinary Medical Center of the University of Tokyo with recurrent hypoglycemic collapse. Clinical findings included proportionate dwarfism, a retained puppy-like hair coat, retained deciduous teeth, and open fontanelles. Endocrine workup demonstrated insulin-like growth factor 1 (IGF-1) below the detection limit, with normal thyroxine and cortisol, and a GH stimulation test confirmed isolated GH deficiency. The pituitary gland was structurally normal on magnetic resonance imaging. Sanger sequencing of GH1 identified four intronic point variants and a homozygous in-frame 6-bp deletion in exon 5 (c.573_578del). The authors used the PROVEAN algorithm to predict the deletion “could be deleterious.” No other affected dogs were reported. The published control data consist of Sanger sequencing of GH1 exon 5 in a single unrelated healthy Chihuahua.

Methodological limitations of the primary evidence

1. The study describes a single affected animal. ACMG criterion PS4 (prevalence increased in affected individuals over controls) cannot be evaluated from one case.
2. No segregation data are presented. Parents, siblings, and other relatives of the proband were not genotyped. Autosomal recessive inheritance is inferred from the proband's homozygous genotype; this is restatement, not demonstration. Criterion PP1 (co-segregation) is not met.
3. No functional validation was performed. PROVEAN is a single in silico prediction tool and does not, alone, satisfy criterion PS3 (well-established functional evidence). Criterion PP3 (multiple computational lines of evidence) is not met with a single algorithm.
4. Four additional intronic variants in GH1 were identified in the same dog (c.10+35T>C, c.11–99A>G, c.288+15T>C, c.289–47_48del) and were not functionally distinguished. Their potential contribution to splicing, expression, or phenotype is undetermined.
5. Whole-exome or whole-genome sequencing was not performed. Causal variants elsewhere (IGF1, IGF1R, GHR, POU1F1, PROP1, LHX4, etc.) cannot be excluded.
6. Allele frequency in the general Chihuahua population cannot be estimated from one healthy control. Criterion PM2 (absent or rare in population databases) cannot be evaluated.
7. The clinical signs reported overlap substantially with normal variation in Chihuahuas and other toy breeds. Open molera is a breed-standard feature of the Chihuahua. Retained deciduous teeth and juvenile hypoglycemia are common in toy breeds and are not typically monogenic.

Part 2. Post-Publication Status (2020–2026)

In the more than five years since publication, no follow-up peer-reviewed study has appeared that (a) identifies a second affected dog with the homozygous genotype and matching phenotype, (b) conducts pedigree segregation analysis, (c) reports functional validation in vitro, or (d) publishes a breed-wide allele frequency estimate.

The Online Mendelian Inheritance in Animals (OMIA) entry for this variant (OMIA:001473-9615) classifies it as “likely causal” and includes the standard OMIA disclaimer that “inclusion of a variant in this table does not automatically mean that it should be used for DNA testing.”

In parallel, the variant has been incorporated into the breeder-decision panels of Embark Veterinary, Labogen, and Genimal, and results are reported to breeders as actionable “Carrier” or “At Risk” findings. Consumer-facing language notes that “further research is needed to determine the full effects of this variant” and that associated issues “are present across multiple toy breeds and may not be caused by this variant,” but the result is tiered alongside validated tests (MDR1, CDDY/IVDD, PRA-prcd, DM) without a clear evidentiary distinction.

Part 3. Empirical Case Series

This section presents empirical data from an independent Chihuahua breeding cohort of 113 dogs, 41 of which have been tested by Embark Veterinary for the GH1 c.573_578del variant. The analysis is derived from Embark genotype results combined with direct owner and breeder reports of each dog's adult phenotype. Older dogs not tested for this variant (n = 37) are excluded from the genotype analysis. The remaining 35 dogs in the broader cohort have phenotype data used in previous comparative analyses.

Two independent asymptomatic homozygous ("At Risk") dogs

Of 41 dogs in this cohort tested for the GH1 c.573_578del variant, 2 are homozygous ("At Risk") — Andy, an actively-breeding stud from the author's program, and Sugarbaby, a pet dog produced by an independent breeding program and placed in a pet home. Both are documented, phenotypically normal adult Chihuahuas from two independent kennels.

Clinical parameter	lio 2020 index case	Andy (At Risk)	Sugarbaby (At Risk)
Genotype (GH1 c.573_578del)	Homozygous	Homozygous	Homozygous
Adult body weight	0.88 kg / 1.94 lb at 6 mo	6 lb adult	4.94 lb adult (4 lb 15 oz)
Ratio vs. lio case	—	3.1× larger	2.5× larger
Open fontanelle / molera	Open fontanelles	Closed	Small, barely noticeable
Retained deciduous teeth	Present	Not reported	None
Hypoglycemia episodes	Severe, every ~3 days	None reported (multi-year vet record)	None
Puppy-like coat retention	Present	Not reported; normal adult coat	Not reported; described as normal
IGF-1 level	Below detection limit	Not measured (clinically well)	Not measured (clinically well)
Growth phenotype	Proportionate dwarfism	Normal size adult	Normal size adult
Health issues	GH deficiency, collapse	None	None
Reproductive function	Unknown (young proband)	Actively breeding; normal offspring	Never bred (produced in a breeding program and sold as a pet)
Formal health testing	GH stim failed, IGF-1 zero	Passed all health testing	No health issues reported by breeder

Neither homozygous dog in this cohort exhibits any of the five clinical signs the lio 2020 paper associated with the GH1 c.573_578del genotype. The two dogs come from two different kennels, were tested independently, and their phenotype information was collected from two different sources (direct owner report for Andy; independent-kennel breeder report for Sugarbaby).

Cohort-wide empirical analysis

Genotype distribution in the tested cohort

Of 113 dogs in the breeding cohort, 41 have been tested for the GH1 c.573_578del variant (37 are older dogs tested before Embark added the variant to its panel). Among those 41 tested:

- 22 Clear (no copies of the variant)
- 17 Carrier (one copy) — 41.5% of tested dogs
- 2 At Risk (two copies) — Andy and Sugarbaby, both phenotypically normal

The observed allele frequency is $q = 0.256$. Under Hardy-Weinberg equilibrium, an allele at this frequency with full penetrance (as a pathogenic recessive variant would show) would produce approximately 6.6% homozygous affected dogs in an unselected breeding population — approximately 2.7 affected dogs among 41 tested. The actual number of clinically affected dogs observed in this cohort is zero.

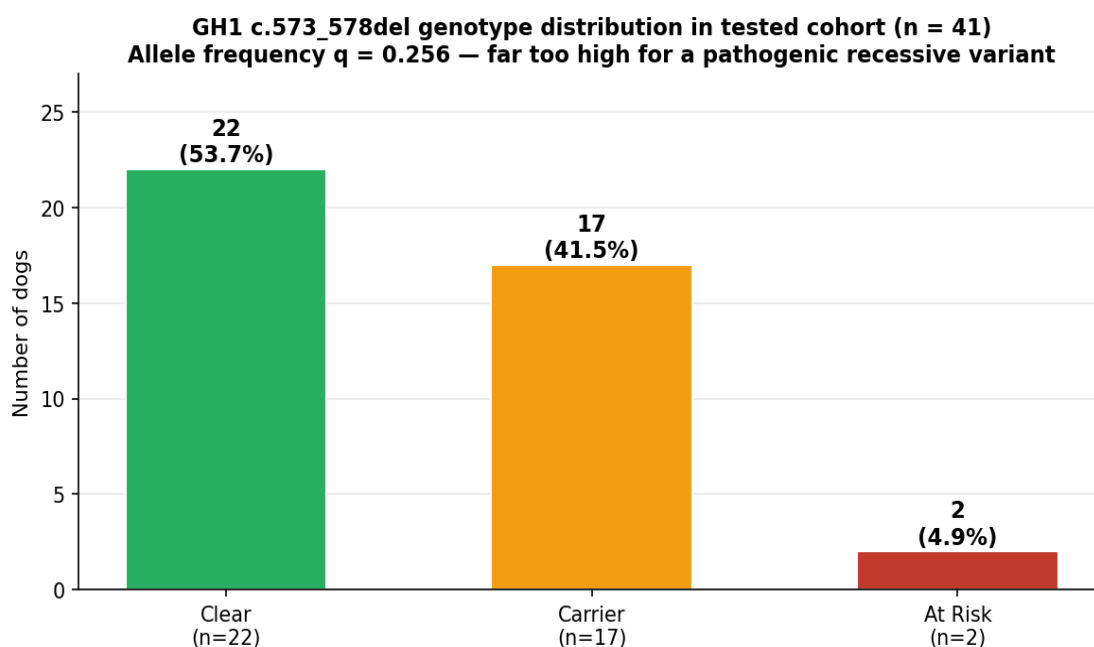


Figure 1. GH1 c.573_578del genotype distribution in 41 tested dogs. Both At Risk dogs are phenotypically normal breeding adults.

Body weight comparison across genotypes

If the c.573_578del variant caused proportionate dwarfism, At Risk (homozygous) dogs should be dramatically smaller than Clear dogs. In this cohort, they are not.

Genotype	n	Median weight (lb)	Mean weight (lb)	Range (lb)
Clear	15	6	5.65	3 – 8
Carrier	8	5	5.12	4 – 7
At Risk	2	6	5.47	4.94 – 6

Kruskal–Wallis test across the three genotype groups: $p = 0.698$. Mann–Whitney U test, Clear vs. At Risk: $p = 0.819$. Neither approaches statistical significance; there is no detectable effect of the variant on body weight in this cohort.

Notably, the smallest dog in the entire tested cohort (3 lb) is a Clear dog. Both At Risk homozygotes are larger than this dog. Every dog in the tested cohort, regardless of genotype, is substantially larger than the lio 2020 index case (1.94 lb at 6 months of age); zero dogs meet the lio severity threshold.

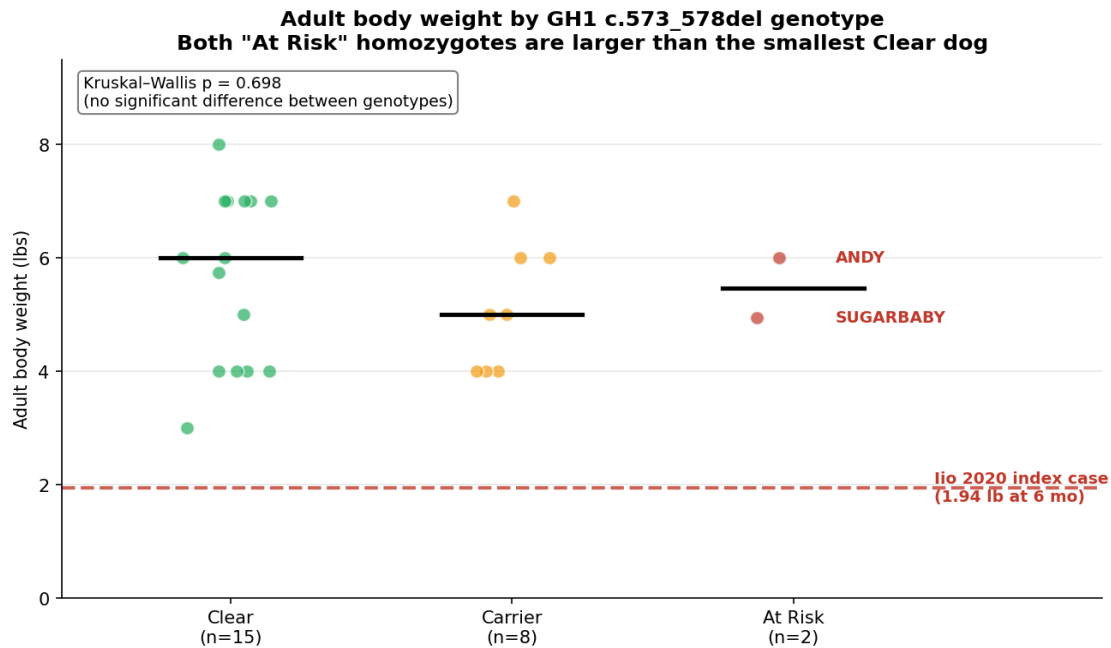


Figure 2. Adult body weight by genotype. Each dot is one dog; black bars are medians. Andy and Sugarbaby — the two homozygous At Risk dogs — are labeled. The lio 2020 index case weight (1.94 lb at 6 months) is marked as a dashed red line.

Clinical phenotype comparison

The lio 2020 paper identified five clinical signs in the index case: (1) proportionate small body size with growth failure, (2) open fontanelles, (3) retained deciduous teeth, (4) retained puppy-like (lanugo) coat, and (5) episodic hypoglycemia. Neither of the two homozygous At Risk dogs in this cohort exhibits any of these signs.

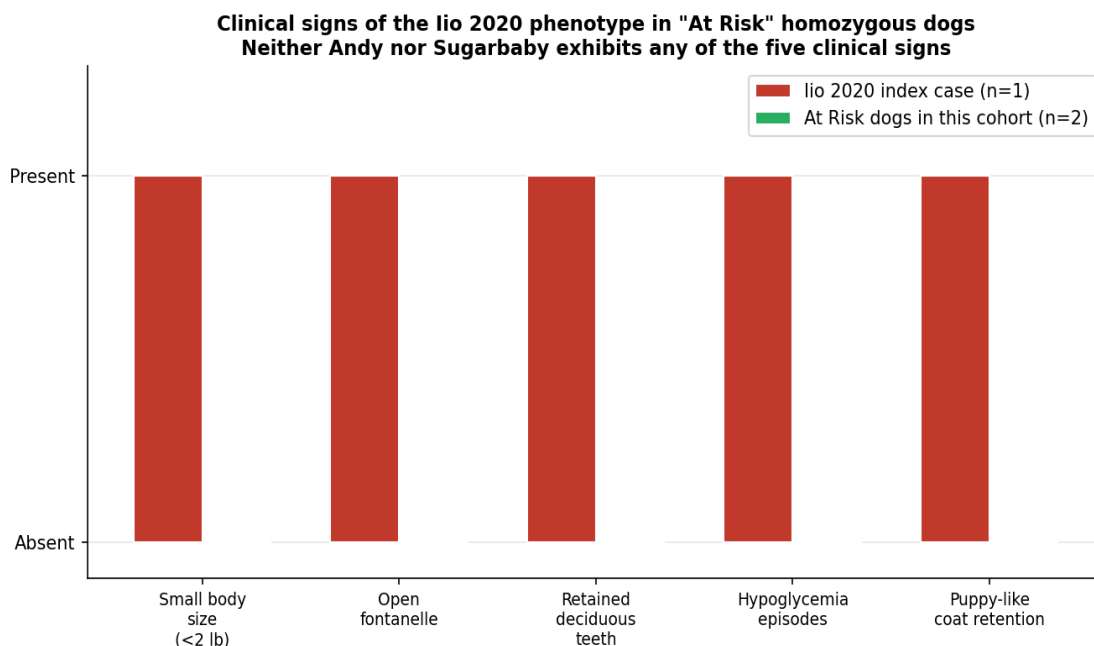


Figure 3. Clinical signs comparison. All five lio 2020 phenotype signs are present in the index case and absent in both homozygous At Risk dogs in this cohort.

Part 4. ACMG/AMP Classification

Applying the ACMG/AMP 2015 framework to the full body of available evidence (published data plus the empirical case series):

Criterion	Meets?	Assessment
PS3 (functional evidence)	No	No in vitro or in vivo functional studies have been performed.
PS4 (prevalence in affected > control)	No	Only one published affected case; no formal prevalence comparison.
PM2 (absent or rare in controls)	No	Allele frequency $q = 0.256$ in this 41-dog tested cohort. Not rare.
PP1 (co-segregation)	No	No family segregation data published; active contradiction from two unrelated asymptomatic homozygotes.
PP3 (computational evidence)	Weak	Single PROVEAN prediction; criterion requires multiple converging algorithms.
BS2 (observed in healthy adult homozygote)	YES — TWICE	Andy (6 lb adult, multi-year clean vet record, actively breeding, closed molera) and Sugarbaby (≈ 4.94 lb adult, no clinical signs, independent kennel, pet) are both homozygous and phenotypically normal.
BS3 (functional)	Implicit	No experimental in vitro evidence; however, in

evidence against pathogenicity)		vivo absence of phenotype in 2 adult homozygotes is a biological functional test in itself.
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ACMG/AMP 2015 guidance: A single BS2 observation is generally sufficient to preclude Pathogenic or Likely Pathogenic classification. Two independent BS2 observations, in the absence of any satisfied pathogenic criteria, support reclassification to Likely Benign. The variant should be classified, at minimum, as a Variant of Uncertain Significance. The balance of available evidence — zero satisfied pathogenic criteria, two BS2 observations, absent functional data, absent segregation data, no replication in five years — supports reclassification to Likely Benign pending formal validation work.

Part 5. Minor Concerns

- The abstract characterizes the deletion as one that “could be deleterious to the function of GH1.” This conditional phrasing, appropriate to a PROVEAN prediction, is not preserved in downstream commercial product communication.
- The lio paper reports no response-to-treatment data (e.g., exogenous GH, levothyroxine). In canine pituitary dwarfism, a positive response to hormone replacement is standard supporting evidence; its absence in the primary literature is a gap.
- The proband was female; no discussion of potential sex-limited expressivity is offered, though the inheritance is claimed to be autosomal recessive.
- The PROVEAN numerical score is not reported in the manuscript text, only the categorical interpretation.

Part 6. Recommendations

8. Reclassification of GH1 c.573_578del. Under ACMG/AMP 2015 criteria the variant should be reclassified from “likely causal” (OMIA) / implicitly-pathogenic (commercial panels) to Likely Benign, pending formal validation.
9. Commercial panel adjustment. Commercial laboratories reporting this variant should either (a) move the result to a clearly labeled investigative or research-use tier, or (b) withdraw the variant from the breeder-decision panel until replication and functional data are published.
10. Breeder communication. Consumer-facing language should preserve the severity threshold of the lio index case (failed GH stimulation test, IGF-1 below detection, collapse-level hypoglycemia at 6 months of age) and explicitly distinguish that phenotype from common, normal-range toy-breed findings (open molera per breed standard, surgical retained deciduous teeth, normal toy-breed juvenile hypoglycemia).
11. Published validation. A breed-wide Chihuahua allele frequency study, a functional assay of the variant in a mammalian expression system, and a prospective case series of clinically affected dogs remain necessary. The empirical analysis presented here is a preliminary step in this direction.

Limitations of This Critique

- The case series presented here is drawn from a single breeder's cohort plus one independent-kennel report. It is preliminary evidence, not a substitute for a formal multicenter validation study.
- Of the 41 tested dogs, full phenotype data (body weight matched to genotype) are available for 25 dogs; matching between Embark records and breeder records was not possible for all dogs.
- Molera, retained dentition, and coat phenotype are not uniformly measured in the broader cohort; the two At Risk dogs have this data by direct inquiry, but broad statistical comparison on these signs is limited by sample size.
- This critique does not exclude the possibility that, in other dogs or in other genetic backgrounds, the variant contributes to a disease phenotype — only that, on the currently available evidence, it does not meet the evidentiary threshold for pathogenicity and should not drive breeding decisions.

References

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