

GDCA IRISH SPOTTING TEST VALIDATION:

Exploration and Validation of Irish Spotting in the Great Dane: Phenotype vs. Genotype

Rationale and Objectives:

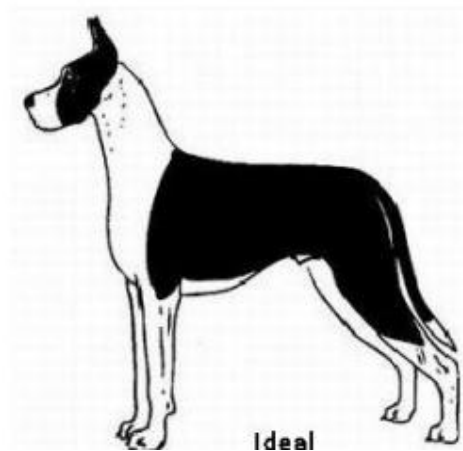
This prospective study recruited owners of Great Danes who show significant white markings to submit photos and samples from these dogs to: (1) assist the Veterinary Genetics Laboratory at UC Davis in validation of their MITF Promoter Length Polymorphism Test; (2) provide owners with information useful for future breeding decisions; and (3) support development of educational material on the topic of coat color/pattern inheritance to be shared with members and non-members of GDCA.

The primary objective was to provide VGL with samples of genetic material from members of the harlequin family (and other Great Danes with significant white markings in locations typical of the Irish pattern) to enable validation of their laboratory test. Secondary objectives were to explore the relationship between phenotype and genotype by examining the relationship between location and size of white markings (via photographs) versus base pair (bp) length via VGL genetic test.

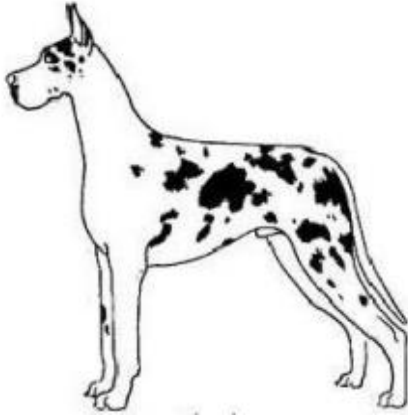
Background:

Irish Spotting describes a distinctive pattern of white markings seen in the black mantle, merle mantle and the harlequin Great Dane. These include a white muzzle with or without a blaze, partial or full white collar, white chest and underside, white on the forelegs and hind legs and a white-tipped tail. The Irish pattern is described in the GDCA Standard and considered highly desirable and worthy of preservation.

Irish Pattern on Mantle



Irish Pattern on Harlequin



Ideal

Irish Spotting has been assumed to be inherited in an autosomal recessive fashion. It “breeds true” in the sense that offspring from litters where both parents of a litter display the classic Irish pattern will inherit and display the pattern depicted above, although minor variations are common.

Historically, this pattern was attributed to specific alleles, s^i , s^p or possibly s^w , located at the S locus (Little, 1957). More recently, however, Irish Spotting has been described instead as a variable length polymorphism in the promoter of microphthalmia-associated transcription factor (MITF). MITF is co-localized with the white spotting locus, S and encodes a transcription factor that controls the development and migration of melanocytes. The expression of MITF protein plays a major role in coat pigmentation (Karlsson et al. 2007). Genetic testing has shown that those dogs with a longer fragment in the promoter, but lacking the mutation that causes piebald, have an observable Irish Spotting pattern. (*See comment regarding piebald below). Data from Korberg et al. 2014 suggests that the composition of the polymorphism may also play a role in the pattern.

Preliminary analysis of genetic sequencing of approximately 3500 Great Danes in the VGL database has revealed as many as five alleles in the MITF promoter that may be involved in determining the appearance (phenotype) of the pattern of white on these dogs. These are expressed as the length of the variable fragment in the MITF promoter as base pairs (bp). In Great Danes tested thus far, the following combinations have been seen: 31/31 (41%), 31/35 (16.5%), 31/36 (8.1%), 35/35 (14%), 35/36 (12%), 36/36 (<1%), and 33 and 34, each with <1%). Unfortunately, photographs are not available for the previously tested Great Danes so it's not possible to correlate these genetic results with specific coat color or variation in white spotting or pattern in those individuals.

Karlsson et al. reported that base pair lengths of 32 bp or less do not appear to be associated with the Irish Spotting phenotype whereas numbers 35 bp or greater have been shown to be associated with the Irish Spotting phenotype. VGL needs additional data on these combinations. If there's correlation of these with phenotype (based on photos), VGL will have evidence their genetic test is, in fact, able to accurately predict the appearance of the dog.

*Note: Unlike Irish Spotting which produces a symmetrical "mantle-like" pattern of markings, less symmetrical white spotting, often called piebald, parti, or random white, is present in many breeds. A specific variant in MITF is associated with piebald spotting and is the basis of commercial tests for piebald offered by several genetic testing labs.

Irish Spotting alleles act by restricting the areas where color (e.g., black, merle or patches in the harlequin) may appear on the dog. Great Danes who inherit the Irish Spotting allele from both parents are expected to display this pattern and reliably produce it in their offspring. Danes who do not have the genotype will appear as solid colors, piebald, almost entirely white or various "mismarks".

Uncertainty arises when Great Danes display some, but not all of the white markings or where there are large breaks in the mantle or other partial Irish pattern. These can arise when one parent is a solid color ("mixed color breeding"), when one or both parents are mixed color bred or have unusual markings in the pedigree. Knowing and/or confirming the genotype can help guide future breedings and increase the probability of Irish patterned offspring.

Until recently there has not been a commercial test for Irish Spotting. Researchers at the University of California Davis Veterinary Genetics Laboratory (VGL) have developed a test, but it is not fully validated. The variable region attributed to Irish spotting has been roughly characterized, but extensive phenotype and genotype data are required to tease out the correlation between the two. This project will collect samples for genetic testing along with photographs in order to confirm the genotype of dogs who appear to be Irish marked and to determine the genotype of others with unconventional markings.

Methods:

Participant Great Danes were recruited via advertisements on the GDCA website, Facebook, letters to GDCA affiliate clubs, flyers at specialty shows and direct appeal to owners.

Owners were asked to provide identification, demographic information and genetic samples similar to that collected on the present VGL Coat Color Test request form. In addition, clear, full body photographs of both sides of the dog and a signed consent form from the dog's owner agreeing to use of the test result and photographs were required.

Great Danes already on record with a piebald test at VGL were also enrolled, provided they submitted the required identification, demographic data and photographs and consent for release of their VGL test report.

A database was created, assigning a unique identifier to each Great Dane enrolled in the study, along with accession numbers assigned to the right and left view photographs, as well as demographic and other information collected on the Irish Spotting –MITF test order form.

Inclusion Criteria (must meet *all* of the following criteria):

Great Dane, including the following:

Harlequin, black, merle, other colors with significant white markings

Owner provided requested dog identification data

Owner provided acceptable photographs of both sides of dog showing white markings

Owner completed and signed a consent form providing permission for GDCA/study team to receive test results and to use dog's information and photographs for study purposes.

Exclusion Criteria (subjects must meet *none* of the following criteria):

Solid color Great Dane

References:

Little, CC. The inheritance of coat color in dogs. 1957. New York, Howell Book House

Korberg IB et al. 2014. A Simple Repeat Polymorphism in the MITF-M Promoter Is a Key Regulator of White Spotting in Dogs. PLOS One: Volume 9 | Issue 8 | e104363

Karlsson EK et al. 2007. Efficient mapping of mendelian traits in dogs through genome-wide association. Nat. Genet. 39(11): 1321-1328. doi: 10.1038/ng.2007.10. Epub 2007 Sep 30.

Results:

A total of 201 Great Danes enrolled in the study. Of those, 197 received and submitted a cheek swab sample to UC Davis Veterinary Genetic Lab (VGL) for testing for MITF-LP, a test proposed to correctly identify/discriminate those with the genotype most closely associated with the Irish Spotting pattern. Despite contacting owners several times, three dogs failed to submit a sample for testing. One dog died before his sample could be collected. Thus, the results below include 197 of the 200 dogs planned for this research.

Demographics of Study Participants

Gender:

Male: 83/197 (42.1%) Female: 114/197 (57.9%)

Age:

Range: 0.14 years to 11.07 years. Average: 3.98 years

Country (of Owner):

Country	Number of Dogs
United States	150
United Kingdom	28
Australia	8
Canada	4
Belgium	3
New Zealand	3
Denmark	1
Total	197

Study Participants, by Color/Markings:

Color/Markings	Number	% of Participants
Harlequin	100	0.51
Black Mantle	52	0.26
Merle Mantle	17	0.09
Fawn or Brindle Mantle	3	0.02
Black & White/not mantle	6	0.03
Black (solid/showable as black)	5	0.03
Piebald (black or merle)	6	0.03
Piebald (brindle or blue)	2	0.01
Other*	6	0.03
Total	197	1.00

*Other includes harlequin with fawn patches (1), harlequin with brindle patches (1), solid merle (1), merlequin (1), white (1), chocolate (1)

Color Test Results:

VGL performed the color/pattern tests using only the information owners provided on VGL's test request form. They did not have access to photos of the dogs. These were provided to VGL after lab testing and used to confirm that color/pattern, as reported by the owner, was consistent with photos of the dog.

1. Harlequin Gene:

Do all dogs listed as harlequin by the owner have one copy of harlequin gene? Yes. All 100 tested as N/H.

Do any dogs listed as merle (N=23) have the harlequin gene? None of the 17 mantle merles had the harlequin gene (N/N). Of the remaining six which included merle piebalds, merlequins, etc., three had harlequin gene (N/H), three did not (N/N).

Of those listed as black mantles (N=52), 40 had one copy of the harlequin gene (N/H), 12 did not (N/N).

Interestingly, all dogs listed as black (N=5) had a harlequin gene (N/H). These appeared to be blacks from mixed color breedings.

Of 28 with “unconventional” colors, 19 had harlequin gene (N/H) and nine did not (N/N). Of the six listed as “black with white markings”, one had harlequin gene (N/H) and 5 did not (N/N).

Harlequin Gene vs. MITF-LP Score:

Harlequin Test Result	MITF-LP Score						Total/ (%)
	31/31	31/34	31/35	31/36	35/35	35/36	
N/N	1	0	7	2	17	12	39 (19.8)
N/H	9	1	24	8	51	65	158 (80.2)
Totals	10	1	31	10	68	77	197 (100)

*Based on VGL Harlequin test result (column AF in spreadsheet)

2. Piebald gene:

Of the 100 which owners identified listed as harlequins, 60 (60%) had no copies of piebald while 38 (38%) had one copy. There were two who tested as S/S (two copies of piebald), however upon inspection of the photos, these were a harl headed white and a mantle headed white. Thus, the owners had mis-identified these piebalds as harlequins.

Of the 52 black mantles, 30 (58%) had one copy of piebald (N/S) and 22 (42%) did not (N/N). Of the 30 that had one copy of the piebald gene (N/S), all had MITF-LP of either 35/35 or 35/36. Of the other 22 without piebald (N/N), 21 were either 35/35 or 35/36 on MITF-LP length. The deviant case had one copy of the harlequin gene (N/H) and tested as 31/36. On inspection of photos, this dog was black with white on chest and front toes, but lacked the other pattern features of a mantle, so this individual appears to be a color classification issue.

Of 18 merle mantles, 11 (61%) have one copy of piebald (N/S) and 7 (39%) did not (N/N). So, the percentages are nearly the same, whether the dog was a black mantle or a merle mantle.

Every dog identified by the owner as piebald tested as S/S (2 copies of piebald). Two others, identified by the owner as brindle piebald tested as N/N, so these were not genetic piebalds. The photos confirmed neither of these were piebald, so these seemed to be owner error in reporting color.

None of the dogs listed as miscellaneous color (fawn, chocolate, blue, etc.) had a piebald gene.

Piebald Gene vs. MITF-LP Score:

Piebald Test Result	MITF-LP Score						
	31/31	31/34	31/35	31/36	35/35	35/36	Total/ (%)
S/S	0	0	0	0	8	0	8 (0.04)
N/S	0	0	16	0	38	32	86 (45.5)
N/N	10	1	15	10	22	45	103 (54.5)
Totals	10	1	31	10	60	77	189 (100)

*Based on VGL Piebald test result (column AD in spreadsheet)

3. MITF-LP:

Of the five that identified as blacks, 4 tested as 31/35 or 31/36. One tested as 31/31. This was an interesting case because this dog appeared to be a black with some white markings (not a mantle), but upon more extensive color testing, had a gene for black and tan. So, the MITF-LP test did detect a case which would not have been predicted, based on phenotype.

Of the 52 that identified as black mantles, 51 tested as 35/35 or 35/36. The single deviant case tested as 31/36 and is the same dog mentioned above. It is neither a harlequin or a mantle and phenotypically is in-between. So, the MITF-LP did identify a deviant genotype and a color identification error.

The six identified as black with white markings (other than the above), all tested as 31/35 or 31/36 as expected.

Of 17 identified as merle mantles, 15 tested as 35/35 or 35/36. One tested as 31/36 and the other as 31/35. Upon viewing the photos, neither was a true mantle merle, although they had some white markings. Again, the test was able to distinguish these from classic mantle markings.

Taken together, the 66 dogs identified by owners as either black mantle or merle mantle whose phenotype was consistent with the color the owner identified:

- 28 were 35/35 and of these, 10 did not have piebald gene (N/N) and 18 did have piebald (N/S).
- 38 were 35/36 and of these, 16 did not have piebald gene (N/N) and 22 did have piebald gene (N/S).

Thus, among mantles with the expected MITF-LP scores, there did not appear to be a relationship with piebald status (or harlequin gene status).

MITF-LP Results, by Color/Pattern:

Color/Pattern	Number of Cases	MITF-LP Score						Comments
		31/31	31/34	31/35	31/36	35/35	35/36	
Harlequin	100	5	0	20	4	33	38	All 20 cases of 31/35 were N/H on harlequin test. 10/20 had piebald (N/S), 10 did not.
Black Mantle	52	0	0	0	1	20	31	31/36 may really be a very heavily marked harlequin
Merle Mantle	17	0	0	1	1	8	7	31/35 has white on chest and feet. No collar. May be more of a solid merle. Has piebald gene (N/S). No harl gene (N/N). 31/36 has white on chest and toes. No collar. White tipped tail. No piebald (N/N) and no harl gene (N/N)
Fawn or Brindle Mantle	3	0	1	1	0	1	0	35/35 has piebald (N/S) and harl gene (N/H). 31/34 had brindle markings, no copies of piebald (N/N), has harl gene (N/H) and also blue dilute (D/d1). 31/35 has fawn markings, has piebald (N/S) and harl (N/H), but also has 2 copies of dilute (d/d) and also black and tan (ay/at).
Black w White/not mantle	6	0	0	5	1	0	0	31/36 appears to be a black with white on chest and white toes. Showable black???
Black (solid/showable as black)	5	1	0	2	2	0	0	31/31 has 237 on merle, no piebald (N/N). Has harl gene (N/H). This dog has white on chest and toes. No collar, no tail tip but small white on face.
Piebald (black or merle)	5	1	0	0	0	4	0	#203-35/35 has merle markings, piebald (S/S) and has harl gene (N/H). #114,197 and 260 were all 35/35, piebald (S/S) and had harl gene (N/H).
Piebald (brindle or blue)	3	2	0	0	0	1	0	35/35 is piebald (S/S), has harl gene (N/H) is blue, but carries brown, per AG testing. Both 31/31s did not have piebald gene (N/N) but did have harl (N/H). These two are from same breeder and are both oddly marked.
Other than above	6	1	0	2	1	1	1	These are odd colors/markings not captured in named categories
Totals	197	10	1	31	10	68	77	

The tabulation above is based on color as reported by owner (column R) in spreadsheet. Comments reveal that in some cases, the color/pattern shown in the photographs was inconsistent with that reported on the VGL color test application.

Discussion:

To achieve validation of a laboratory test requires that the test accurately discriminates between discrete groups of individuals, in this case Great Danes which exhibit vs. those who do not exhibit the specific pattern of white markings defined as Irish Spotting. In other words, does the MITF-LP score match and predict the phenotype of the dog in the absence of other information?

Published literature and data from VGL suggest that MITF-LPs of 35 and 36 are associated with the Irish pattern as seen in Great Danes we call “mantles”. Consequently, we should expect

black mantles and merle mantles to be either 35/35 or 35/36 (there were no 36/36 in this study).

Looking at the group of black mantles above, we see 52 cases. Of these, none were 31/31, 31/34 or 31/35, which is what we would expect. Fifty-one cases tested as either 35/35 or 35/36. There was one 31/36. Upon inspection of photos, this individual has white markings on the feet and chest, but no white on the neck, underside or belly, so it does not meet the definition of Irish Spotting pattern. In general, those dogs with a "31" have some white markings, but not the complete Irish spotting pattern. The test therefore performed as expected. It does not appear that the presence of one copy of the piebald gene influences phenotype in the black mantle. Thus, one could not predict this genotype visually. Although there were few in this study which reported results of the merle test, we did not see evidence that scores below 237 affected the phenotype, judged visually. All six individuals displayed the elements of the Irish pattern.

In the group of merle mantles above, 17 were reported by owners as such. Of these, none were 31/31 or 31/34. There was a single case of 31/35 and one case of 31/36. The 31/35 has a white chest and feet, but no collar or white on the underside of body or inside of legs. The 31/36 individual has white on feet and chest, but no collar or white on underside of body or inside of legs. The remaining 15 were either 35/35 or 35/36. The test therefore performed as expected.

Considering the group of 100 identified as harlequins, MITF-LP results are a bit more ambiguous. Examination of photos revealed some of these were actually piebalds (and confirmed by piebald gene results), while others were actually mismarked blacks or mislabeled mantles.

There were five which tested as 31/31. None of these had Irish markings. Some were spotted dogs without pattern; others had minimal white markings. All had one copy of the harlequin gene (N/H) and all tested negative (N/N) for piebald. There were no 31/34's.

Twenty tested as 31/35. All 20 had one copy of the harlequin gene. Ten had one copy of piebald and 10 did not. Based on their photographs, approximately half of these appeared to have normal Irish pattern. Could these reflect the pseudo-Irish genotype (S/sp) as opposed to the "true" Irish genotype: (si/si)? The other half of this group showed some elements of Irish pattern such as white on chest or feet, but were lacking other elements such as white collar, white on underside or inside of legs. There's a definite trend in this group to have very large areas of black and/or strikingly asymmetry of black markings on left vs. right sides.

Among the 20 harlequins in this group there's also a trend of mixed color parentage involving solid color fawn, black or blue Great Danes. Five of these 20 were from the same breeder with three of these five having merle length of 266. Three dogs which appeared to have normal Irish spotting were sired by the same individual who had mixed color breeding farther back in the pedigree.

Four harlequins tested as 31/36. None of these individuals could be described as classic Irish pattern. As a rule, they exhibited some white markings and usually had large, usually asymmetrical black markings. All were negative for piebald and had one copy of the harlequin gene.

The remaining 71 harlequins were either 35/35 (N=33) or 35/36 (N=38), consistent with what one would expect in harlequins which meet the breed standard. Two of the 35/35 identified by owners as harlequins had two copies of the piebald gene (S/S). Upon inspection, their photos confirmed both cases as piebalds, so the MITF-LP test did discriminate piebald from Irish Spotting pattern as was expected. The piebald gene was distributed fairly evenly among the remaining 31 dogs with MITF-LP of 35/35. Twelve were N/N (no copies of piebald) and 19 had one copy (N/S).

Of the 38 harlequins with MITF-LP of 35/36, none had two copies of the piebald gene. Twenty-nine had no copies of piebald gene (N/N) and nine had one copy (N/S). Interestingly, there were three very lightly marked harlequins in the 35/36 group which some might have assumed to be piebald. Two of these had one copy of the piebald gene. The third one did not. Interestingly, none of these dogs showed merle patches in their photos. Many Great Dane breeders have claimed that all harlequins necessarily have some merle markings. It appears this is not the case.

Discussion:

Although Great Danes with any "31" (MITF-LP of 31/31, 31/34, 31/35 or 31/36) did not display the full set of markings defined as Irish pattern, visual inspection of photographs did not suggest systematic differences in white markings between the groups of dogs with MITF-LP of 35/35 vs. those with 35/36.

Of the 68 cases of MITF-LP of 35/35, all showed white in the areas expected of Irish Spotting pattern. However, there were eight cases where the dog in question was actually a piebald, as confirmed by photos and presence of two copies of the piebald gene (S/S).

Of the 77 cases of MITF-LP 35/36, all showed Irish pattern. There were no piebalds in this group. Is this a clue?

Looking closer at the group of cases with a 31:

Of the 10 cases with MITF-LP of 31/31, all were oddly marked. Some had large, rounded markings like a piebald, but none tested as a piebald (SS). Several carried genes for colors not typical in the harlequin family (e.g., sable, blue, black and tan) so there is evidence of mixed color breeding (MCB) in the pedigree. Several cases were from the same breeder, although not necessarily full or half siblings.

There was only one case with MITF-LP of 31/34. This was a “harlequin” with brindle patches instead of black. This individual was referred to us for testing. Unfortunately, her breeder and pedigree are unknown. She tested as D/d1, K/N, ay/ay.

There were 31 cases with MITF-LP of 31/35. Approximately half of them showed white markings in locations typical of Irish pattern (photographs). Of those whose pedigrees could be located, most were from MCB breeding. Few were tested for merle. Those that had results did not show length <264. Many in this group were from “solid blacks” that carried blue or another recessive). Three, plus another dog from the 31/31 group were from the same sire, who himself was oddly marked.

There were 10 cases with MITF-LP of 31/36. There were no piebalds or piebald carriers in this group. Eight of these carried for harlequin, two did not (N/N). Those two were black with a few white markings on the feet or chest. In general, this group of dogs were heavily marked often with quite asymmetrical markings. All or nearly all dogs in this group were products of MCB. Several carried for unusual colors, e.g., wild sable and showed evidence of MCB with fawn at mask locus E (Em/E) or K (K/N). Several of these ten were from the same two breeders.

Relevance to GDCA Breeder’s Color Code:

Although the primary objective of this project was to validate VGL’s Irish Spotting Test, many of the study’s findings are directly relevant to Great Dane breeders, assuming they have a desire to make decisions consistent with preservation of the Irish pattern and maximizing the number of genetically appropriate breeding partners in the future.

In the past, GDCA endorsed specific breedings with the objective of maximizing the production of offspring having colors and markings which meet the standard. To that end, GDCA defined color “families” as depicted in the figure below:

Color of Dane	Approved Breedings	Desired Pedigrees
1. FAWN 1. BRINDLE	1. FAWN bred to FAWN or BRINDLE only. 1. BRINDLE bred to BRINDLE or FAWN only.	Pedigrees of FAWN or BRINDLE Danes should not carry BLACK, HARLEQUIN or BLUE upon them.
2. HARLEQUIN 2. MANTLE 2. BLACK (HARLEQUIN BRED)	2. HARLEQUIN bred to HARLEQUIN, BLACK from HARLEQUIN BREEDING or BLACK from BLACK BREEDING only. 2. MANTLE bred to HARLEQUIN, MANTLE, BLACK from HARLEQUIN breeding or BLACK from BLACK breeding only. 2. BLACK from HARLEQUIN BREEDING bred to HARLEQUIN, BLACK from HARLEQUIN BREEDING or BLACK from BLACK BREEDING only.	Pedigrees of HARLEQUIN, MANTLE or HARLEQUIN BRED BLACK Danes should not carry FAWN, BRINDLE or BLUE upon them.
3. BLUE 3. BLACK (BLUE BRED)	3. BLUE bred to BLUE, BLACK from BLUE BREEDING or BLACK from BLACK BREEDING only. 3. BLACK from BLUE BREEDING bred to BLUE, BLACK from BLUE BREEDING or BLACK from BLACK BREEDING ONLY.	Pedigrees of BLUE or BLUE BRED BLACK Danes should not carry FAWN, BRINDLE, or HARLEQUIN upon them.
4. BLACK (BLACK BRED)	4. BLACK from BLACK BREEDING bred to BLACK, BLUE or HARLEQUIN and MANTLE. (See note below)	Pedigrees of BLACK BRED Danes should not carry FAWN, BRINDLE, HARLEQUIN, MANTLE or BLUE upon them.

If one adheres to these guidelines, those in the harlequin family should not carry dilute at D locus, should have two copies of dominant black at K locus, and be homozygous for fawn/sable at the agouti (A) locus. Thus, we can expect results of color testing, if done, to be D/D, K/K, ay/ay. Although very small amounts of white markings (toes, front of chest) are allowed on members of the fawn/brindle, blue/blue-bred black or black-bred black color families, these should not have white markings on the face or elsewhere on the body.

Insights from Extended Color Testing:

Less than half of the Great Danes who participated in this study requested extended testing for the above color tests. Nevertheless, results of these tests are instructive and demonstrate the influence of MCB on today's Great Danes and its impact for future choices of breeding partners.

Of 197 Great Danes in the study, 102 (51.8%) underwent testing for color related genes at the D, K or A locus. If one considers only the optimal breeding group in this study, namely, harlequins, black mantles and merle mantles, there are 169 such individuals. Of these, 88 had additional color results available.

Fawn (K locus)

Of the 88 harlequin, black mantle or merle mantle individuals with K locus results, 14 (15.9%) were K/N, indicating they carry for fawn. If we include all study participants with results for K locus, the percentage increases to 21.6% (19/88). This can result either from breeding directly to fawns or to other colors (e.g., black, blue or brindle) which carry a fawn recessive. None of these were evident from the photos but could usually be predicted based on examination of the pedigree.

Dilute (D locus)

Of the 77 harlequin, black mantle and merle mantles with D locus results, 14 (18.2%) were D/d, indicating they carry the gene for dilute color. If one includes all 89 study participants with testing for D locus, the percentage increases to 20.2% (18/89). None of these were evident from the photos but could usually be predicted based on examination of the pedigree.

Agouti (A locus)

Of the 74 individuals with A locus results, 67 (90.5%) had the expected ay/ay genotype associated with fawn color. Of the seven with unexpected results, 4 tested as ay/at, a recessive associated with black and tan coloration, while 3 others tested as ay/aw, a recessive associated with wolf or sable coloration. Five of the seven also had abnormal ("31") results on the MITF-LP test and did not display the Irish pattern. Of those with available pedigrees, all were products of MCB. Of the other two, both were 35/35 on the MITF-LP test and displayed the Irish Spotting pattern. One was a fawn mantle of obvious MCB. The other was a classically marked harlequin and rather surprising, demonstrating the value of testing for all colors included in the Great Dane panel.

Conclusions:

Test Performance:

The primary objective of this study was to provide data to evaluate the performance of VGL's MITF-LP Irish Spotting test. From the perspective of the Great Dane owner/breeder, this means ability to distinguish those possessing the genotype associated with Irish pattern (si/si) which breed true from the "pseudo-Irish" (S/sp) which can appear to be Irish patterned but will not reliably produce Irish patterned offspring. For additional explanation, see: <http://www.Yaresville.com/standard/mantle-coat-colour-genetics>

VGL's MITF-LP test performed well in identifying piebald dogs, even when misidentified by the owners as harlequins, etc. Since we had information on parentage and pedigrees were available for most individuals, it was possible to ascertain mantle individuals likely to be S/sp pseudo-Irish) vs. those expected to be si/si (true Irish). The MITF-LP test was successful in that these tested as either 35/35 or 35/36, however several piebalds tested as 35/35. Thus, further work will be needed to validate the test. More detailed sequencing of the MITF gene showed that MITF-LP lengths of 35 or 36 showed either five (for MITF-LP of 35) or two (for MITF-LP of 36) different allele sequences. This may provide opportunity for further test refinement.

The results of these research efforts validate MITF-LP allelic association of Irish-spotting in the Great Dane that is consistent with observations in other breeds. Genetic testing provides results that identify allelic variants of the Irish spotting locus status independent of the phenotype of the animal. Thus, true Irish spotting can be distinguished from pseudo-Irish spotting resulting from piebald, higher number merle alleles or Great Dane Harlequin. Similarly, dogs with Irish spotting masked by significant white patterning can be identified. This test offers breeders the opportunity to further identify genetic variants contributing to genetic composition in litters. While this information is valuable and a validated test for allelic size determination at MITF-LP is possible, sequence variation of those sizes may be associated with heritable differences in the pattern observed. Additional research is needed to assess sequence level variation of Irish spotting 35 and 36 alleles. Additionally, the 36 variant needs to be further interrogated. Based upon the frequency of the 36 allele in the study population, homozygous 36 individuals should be present, but were not observed. The absence of a homozygous 36 Irish spotting identified individual may represent ascertainment bias, but it is interesting to note that a 38 allele in other breeds do not correspond to any visible white pattern. Thus, there may be more to this locus than a threshold value of 35 correlated with the Irish spotting pattern.

Impact of Mixed Color Breeding:

Traditionally, Great Dane breeders have attempted to maximize the percentage of puppies which correspond to the color and pattern description in the AKC standard. As noted above, the GDCA Breeder's Color Code was written explicitly to endorse specific matings which support that goal and to discourage others. Based on the findings of this study as well as AKC litter

registrations and advertisements, clearly this advice is not being followed, not only by “backyard” and other casual breeders, but also by well-known and successful show breeders. We found at least 15% of harlequin family breeding stock carry genes for one or more undesirable recessive color (fawn, brindle, blue).

Since the average age at enrollment was approximately 4 years, our results reflect breeding practices of the late 2010’s. Judging by litter advertisements and announcements, the practice of MCB is even more prevalent today. This poses a significant reduction in potential future breeding partners if one wishes to maximize the percentage of show-marked puppies in their litters.

At least 12 individuals in the study were phenotypically neither mantle nor solid black or merle. These “in-betweens” typically had white markings on the feet/toes and large patches of white on the chest or front, but without white on the neck or underside of the dog. A few had a white-tipped tail and/or very slight, asymmetrical white markings on the muzzle. All were “31’s” on MITF-LP and all (where a pedigree was available), were products of MCB. Under the current standard, these are neither showable blacks or mantles. In addition to these, several individuals had such asymmetrical markings they appeared to be either mantle or harlequin, depending on which side they were viewed from.

Challenges and Opportunities:

Numerous instances of errors in reporting the color/pattern of their dogs complicated analysis of the data. This was especially true of piebalds identified as harlequins, but also merlequins and black and white mismarks which were neither solid nor mantle. Photographs enabled amendment of color/pattern in this study’s database and resolved some discrepant genetic test results.

These misidentifications were probably unintentional. Nevertheless, they diminish the accuracy of research based on AKC, VGL and other health registry records and can lead to incorrect conclusions.

Suggested Actions:

1. GDCA should add clear definitions and photos of non-standard colors and markings in the Illustrated Standard and in Breeder and Judges’ Education materials.
2. GDCA should provide education to dispel the myth of benefit of the piebald gene, whether as homozygote or heterozygote. The piebald gene does not “clean up color”.
3. GDCA needs to work with VGL and other color test providers to develop clear guidance on color testing and materials which aid owners in interpreting the results. Similarly, owners should be encouraged to take advantage of the entire panel, including extended testing of all options, as appropriate to color.
4. GDCA’s decision to approve showing of solid merles has sent a mixed message which creates confusion and leaves the impression that preservation of Irish pattern is no

longer an important objective. A clear statement on this should be added to GDCA's educational materials.

5. Breedings between harlequins and solid blacks/black mismarks, many of which carry undesirable recessives, decrease the proportion of offspring with true Irish phenotype and genotype. GDCA needs to provide clear guidance on the impact of introducing solid color individuals into the harlequin/mantle breeding pool.

While color and pattern should not take precedence over conformation in judging the Great Dane, the impact of undesirable color and pattern genes needs to be weighted more heavily when making breeding decisions as these impact future options.

Special thanks to the owners and breeders who participated in this research project and to the Great Dane Club of America and the GDCA Charitable Trust for their continued support.

~Mary Anne Zanetos, author.