



## Cerebellar Abiotrophy DNA Test Development at the UC Davis Veterinary Genetics Laboratory (VGL)

By Beth Minnich, Chair of the Research Advisory Panel of the Arabian Horse Foundation with special thanks to Dr. Cecilia Penedo for reviewing this material and Dr. Leah Brault for her notes on the haplotype test  
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The recently announced identification of a mutation associated with Cerebellar Abiotrophy (CA) is an important finding in the ongoing research and has advanced the CA test, now being offered by several labs around the globe. To help provide a better understanding of how the CA test has been developed, the following summary has been prepared.

The Arabian Horse Foundation thanks our donors, along with the owners who have submitted samples, in their ongoing support of this important research effort.

### The Early Work

In 2001, the UC Davis VGL utilized a process known as genome scanning, using samples obtained from the VGL's CA herd, in an attempt to identify which chromosome housed the "CA gene". In simplified terms, whole genome scanning looks for statistically significant associations between DNA markers along each chromosome and a trait being mapped. From 2003 to 2009, research progressed with access to additional samples and the use of valuable new tools developed from the [sequencing of the horse genome](#) (which was completed in 2007). This phase of the research resulted in the 2007 mapping of CA to horse chromosome 2 (ECA2)

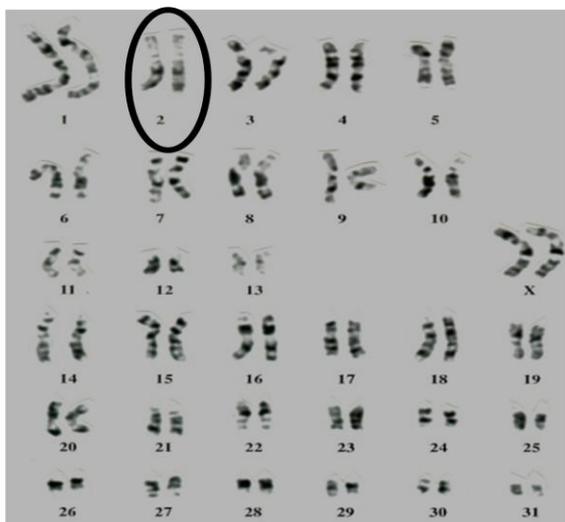


Figure 1: The CA gene has been mapped to horse chromosome 2 (ECA2).  
*Image provided by the UC Davis VGL*

(figure 1). From 2007 to 2009, work continued with [fine mapping in order to narrow the location of the CA gene on ECA2](#) and to develop a set of genetic markers associated with the disease. This set of markers was established which formed the foundation of the marker based DNA test, also known as a haplotype test, which was incorporated into the research project in 2007 and offered to owners commercially in 2008.

### The Haplotype Test

The basis for the haplotype test is that the CA mutation has an ancestor which is the horse in which the original CA mutation first arose. This ancestor had a specific set of alleles (variants) at the DNA markers around the area of the CA mutation. These markers are all close together and the concept is that they will "travel" together with the CA gene.

For example, when a horse passes on the allele responsible for CA, it will also pass along a group of markers that we will oversimplify and identify as “ABCDEF”. When the horse passes on the normal allele, it will also pass along a different group of markers identified, for example, as “GHIJKL”. As such, the CA mutation moves with these associated markers through the generations.

When the UC Davis VGL first started studying known CA affected foals and their parents, who by default were known CA carriers, every affected foal possessed the markers “AA BB CC DD EE FF” and every carrier horse possessed the markers “A\_ B\_ C\_ D\_ E\_ F\_”. Since most of the foals studied early on were fairly closely related, this was not a surprise. The assumption was that the markers were inherited from a shared ancestor relatively up close in their pedigrees. However, as additional samples from affected foals were received for testing, it was noticed that these foals also had the “AA BB CC DD EE FF” markers. Since the newer group of foals did not have any common ancestors within ten generations or so of the first group of foals, this was an indication that CA has a single ancestral source in the Arabian breed. With these affected foals coming from a variety of bloodlines, it indicates the CA mutation predates the division of the various nationality/geographic subgroups within the breed. Thus, making CA an “old mutation”.

The UC Davis VGL identified approximately 40 CA markers and developed the test with 15 of these markers; later including 2 additional markers. Table 1 is an example of the CA haplotype test using 17 markers. This table shows the haplotypes for several horses used in the study, including tested affecteds both with and without clinical signs, carriers and clear horses.

## DNA types and CA status

STATUS	1	2	3	4	5	6	7	8	*9*	10	11	12	13	14	15	16	17
CA/CA	KM	PP	KK	MM	MM	AA	MM	GG	AA	AA	FF	MM	MM	MM	MM	RR	MM
CA/CA	KK	PP	KK	MM	MM	AA	MM	GG	AA	AA	FF	MM	MM	MM	MM	RR	MM
CA/CA	MM	PP	KK	MM	MM	AA	MM	GG	AA	AA	FF	MN	MM	LM	MM	CR	KM
CA/CA	MM	PP	KP	JM	MM	AA	MM	GG	AA	AA	FF	MM	MM	MM	MM	RR	MM
CA/CA	MM	PP	KP	JM	MM	AA	MM	GG	AA	AA	FF	MM	MM	MM	MM	RR	MM
CA/CA	MM	PP	KK	MM	MM	AA	MM	GG	AA	AA	FF	MM	MM	MM	MM	RR	MM
CA/CA	MM	PP	KK	MM	MM	AA	MM	GG	AA	AA	FF	MM	MM	MM	MM	RR	MM
CA/CA	MM	PP	KK	MM	MM	AA	MM	GG	AA	AA	FF	MM	MM	MM	MM	RR	MM
CA/CA	LM	HP	KK	MM	MM	AA	MM	GG	AA	AA	FF	MM	MM	MM	MM	RR	MM
N/CA	<u>IM</u>	<u>PP</u>	<u>HK</u>	<u>LM</u>	<u>JM</u>	<u>AT</u>	<u>LM</u>	<u>GT</u>	<u>AG</u>	<u>AG</u>	<u>EK</u>	<u>MN</u>	<u>MO</u>	<u>LM</u>	<u>MN</u>	<u>RT</u>	<u>MN</u>
N/CA	<u>KM</u>	<u>HP</u>	<u>KM</u>	<u>JM</u>	<u>MM</u>	<u>AT</u>	<u>IM</u>	<u>GT</u>	<u>AG</u>	<u>AG</u>	<u>FQ</u>	<u>MM</u>	<u>MM</u>	<u>LM</u>	<u>MM</u>	<u>KR</u>	<u>MO</u>
N/CA	<u>IM</u>	<u>MP</u>	<u>KN</u>	<u>IM</u>	<u>JM</u>	<u>AT</u>	<u>LM</u>	<u>GT</u>	<u>AG</u>	<u>AG</u>	<u>ET</u>	<u>MN</u>	<u>MP</u>	<u>LM</u>	<u>MO</u>	<u>KR</u>	<u>MM</u>
N/N	KL	HM	HN	II	JJ	TT	LL	TT	GG	GG	KT	NN	OP	LL	<u>MN</u>	CK	<u>KM</u>
N/N	<u>IM</u>	<u>IP</u>	HQ	IL	U	<u>AT</u>	<u>LM</u>	TT	GG	GG	KK	NN	OO	LL	NP	QT	<u>MN</u>
N/N	<u>KM</u>	PP	HH	IL	JJ	TT	LL	TT	GG	GG	KT	NN	OP	LL	<u>MN</u>	CT	KN

Table 1: DNA types from several horses in the study and their related CA status. The highlighted area indicates a refinement of the location of the CA gene.

*Image provided by Dr. Cecilia Penedo*

Results for this test were reported as follows\*:

- **N/N**: Normal. Horse does not possess markers associated with CA.
- **N/CA**: Carrier (1 copy of gene). Horse has markers associated with CA and is considered to be - phenotypically normal and a carrier of the disease gene.
- **CA/CA**: Affected (2 copies of gene). Horse has markers associated with CA and is considered to have the disease.

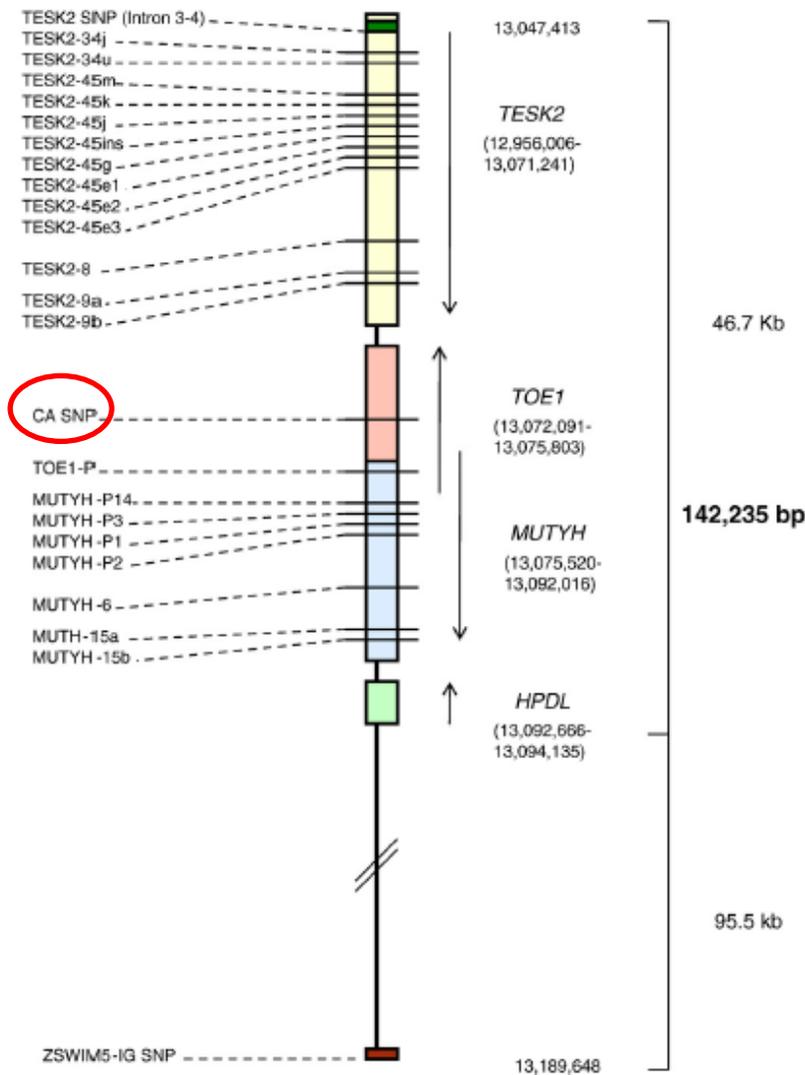
\*There were a few cases of horses testing with an “odd haplotype”, meaning that the horse had some, but not all, of the CA makers. In these cases, the results were initially listed as “undetermined”. As the CA region continued to be narrowed down even further, these “undetermined” cases were resolved and re-classified with a specific result.

While this test was not a definitive test, it was the only tool available to assist owners in making breeding decisions regarding CA; to help avoid the mating of two carriers. Over the past several years, there has been discussion on the merits of offering a marker based DNA test vs. waiting until a mutation is identified and a direct DNA test is developed. In reality, the marker based DNA test for CA gave owners an option, while research continued to identify the causative mutation for CA.

### The Mutation

In the [February 2011 issue](#) of the journal, *Genomics*, the Penedo group published a paper announcing the identification of a mutation associated with CA (*Figure 2*). This modification of the normal genetic code involves a single nucleic acid base change. The normally found nucleic acid, guanine (G), has been changed to adenine (A). This coding change results in the production of a different amino acid; the normally produced amino acid, arginine, is replaced with histidine.

Of the four genes located in the CA region, two of them, *MUTYH* and *TOE1*, have some brain involvement. Although *TOE1* is not expressed at high levels in the cerebellum, the *MUTYH* gene is involved in the repair of Purkinje cells. With the ends of these two genes partially overlapping one another, it is possible that the mutation influences the regulation of *MUTYH*. Of note, specialized analysis of cDNA from the cerebella of CA affected and unaffected horses indicates that the *MUTYH* gene is not fully functioning in affected horses.



**Figure 2: Region of ECA2 identified as containing the potential causative mutation for CA.**

*Source: L.S. Brault et al. / Genomics 97 (2011) 121–129*

Due to the wide range of expression that has been reported by owners among horses tested as CA/CA (affected), it is possible that, in some cases, horses could have the affected genotype and show no clinical signs. If the MUTYH gene is not fully functioning, this could explain the variable expression. The physical differences between CA/CA (affected) horses could be due to varying levels of MUTYH function, resulting in differing amounts of Purkinje cell damage, between individual horses. Alternatively, it is also possible that a small number of horses carry a suppressor mutation that can assist in compensating for the effects of the disease.

Because of the unusual location of this potential causative mutation for CA, the VGL is conducting further study of these two candidate genes.

### **Retesting**

As part of the work to further develop the DNA test for CA, all horses tested at the VGL with the marker based version of the CA test were retested with the recently identified mutation. Although the marker test had a high rate of accuracy, it was expected that, in a few exceptional cases, it might incorrectly indicate the presence of the CA gene. Results from that retesting showed that the marker based test was 97% accurate in relation to the new version of the test which now incorporates the mutation. While no horses were changed from N/N (clear) to N/CA (carrier) or CA/CA (affected), of the 491 horses retested, there were a small number of changes. Fifteen (15) horses previously tested as N/CA were determined to be N/N and one (1) foal tested as CA/CA, who has shown no clinical signs of CA, has been determined to be N/CA. With the identification of a mutation associated with CA, the genetic test for CA is now firmly established as an accurate test to be used for determining clear, carrier or affected status of foals and horses to be used for breeding.

***NOTE: Results from testing done at the UC Davis VGL are completely confidential and are not shared by the lab with any of the public reporting lists; only owners can make the decision whether or not to post their test results.***

***More detailed information on the CA Research Project can be found at <http://www.arabianhorsefoundation.org/news.html>***