Proc. Assoc. Advmt. Anim. Breed. Genet. 25: 238 - 241

INVESTIGATING THE GENETIC CAUSE OF WRY FACE IN AUSTRALIAN JERSEY CATTLE

C.J. Vander Jagt[1], C.M. Reich[1], I.M. MacLeod[1,2], M.E. Goddard[1,3], B.J. Hayes[4], T.T.T. Nguyen[5], G.J. Nieuwhof[5] and A.J. Chamberlain[1,2]

[1]Agriculture Victoria, Centre for AgriBiosciences, Bundoora, VIC, 3083, Australia
[2]School of Applied Systems Biology, La Trobe University, Bundoora, VIC, 3083 Australia
[3]Australia Faculty of Veterinary & Agricultural Science, The University of Melbourne, Parkville, VIC, 3052, Australia
[4]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, St Lucia, QLD, 4072, Australia
[5]DataGene Ltd, Centre for AgriBiosciences, Bundoora, VIC, 3083, Australia

SUMMARY

Wry Face (WF) is a mammalian condition resulting in facial asymmetry. It is most obvious in long-faced species (e.g. horses and cattle) and ranges in severity. A mild hereditary form of WF is seen at a low frequency in the Australian Jersey cattle population. To investigate the underlying genetics and mode of inheritance of WF, a pilot study was performed. Four WF Australian Jersey cows and one unaffected half-sibling were whole genome sequenced (WGS) and included in Run 9 of the 1000 Bull Genomes Project (1kbulls). A subset of genetic variants found in the WF cows compared to the unaffected half-sibling were in or near genes associated with disorders involving facial deformities. This study is being expanded to validate these results and increase the power to detect more potential WF causal variants. Identifying WF causal variants and including them in routine DNA testing may allow farmers to avoid high risk matings that could result in WF offspring.

INTRODUCTION

WF is a mammalian condition causing facial asymmetry. WF is typically congenital, resulting in maxilla deviation and sometimes involves the mandible. It is most obvious in long-faced species such as horses and cattle (Abdelhakiem and Elrashidy 2017). The condition ranges in severity from a slight < 5^{o} lateral deviation, only impacting aesthetics, to severe > 60^{o} lateral deviation impacting breathing and feeding (Aiello and Moses 2016). Individuals with severe WF often do not survive to adulthood. A mild hereditary form of WF is seen at low frequency in the Australian Jersey cattle population, but at high frequency within some herds (mode of inheritance is unclear). This form of WF does not appear to impact quality of life or production of affected cattle.

Despite the incidence of WF in cattle, there have been few studies examining the inheritance mode or underlying genetics. Here we present a small study examining WGS from four WF Australian Jersey cows (two different herds) and one unaffected half-sibling cow. We demonstrate that we can identify candidate genetic variants and genomic regions which may underlie WF. We suggest that it would be advantageous to expand this study to validate these results and increase the power to determine the inheritance mode and detect the most likely candidate causal variants.

MATERIALS AND METHODS

Tail hair samples were obtained from four Australian Jersey cows that had been visually assessed as having WF by conformation classifiers and one unaffected half-sibling Australian Jersey cow (two different herds). DNA was extracted using the DNeasy Blood and Tissues Kit (Qiagen) and WGS libraries prepared with the NEBNext Ultra II DNA Library Prep Kit. Libraries were sequenced in a 150 cycle paired-end run on a NovaSeq6000 (Illumina). Raw sequence reads were processed

according to the 1kbulls guidelines and submitted for inclusion in 1kbulls-Run9 (reviewed in Hayes and Daetwyler 2019).

Jersey cattle genotypes in 1kbulls-Run9 were obtained (179 individuals) and filtered for variants that were homozygous for the reference allele in the unaffected half-sibling and either heterozygous or homozygous for the alternate allele in all four WF cows. Assuming there may be more WF individuals in 1kbulls unknown to us (at low frequency), further filtering was applied so that the alternate allele would be seen in ≤10 individuals (~5% of individuals). Remaining variants were run through a haplotype detector (custom in-house program) and annotated using Ensembl Variant Effect Predictor (VEP) (McLaren *et al.* 2016). *Bos taurus* genes identified by VEP as being associated with these variants were included in over-representation analysis of GO Biological Processes (BPs) and KEGG pathways using DAVID (Huang *et al.* 2009). MalaCards (Rappaport *et al.* 2013) was used to determine if these genes had an association with human diseases affecting craniofacial skeletogenesis and/or other disorders involving maxillofacial dysmorphism.

To investigate the WF inheritance mode, pedigree information for the four WF cows was provided by DataGene Limited and interrogated to identify common ancestors (manually and using custom scripts). The pedigree was visualised using the R package visPedigree (Luan 2018).

RESULTS AND DISCUSSION

Pedigree analysis revealed all five cows could be traced to two common ancestors: "Secret Signal Observer" (paternal line) and "Soldierboy Boomer Sooner of CJF" (combination of maternal and paternal ancestry) (Figure 1). However, we cannot confirm that either bull had WF as there are no phenotypic records and only side-profile photographs available. In an early study by Ewing (1957) examining frequency (21.5%) and inheritance mode of "twisted face" (presumably WF) in a North American Jersey cattle herd, it was concluded that "twisted face" was most likely a simple recessive trait. Our pedigree cannot definitively corroborate this conclusion as there is an insufficient number of animals and inadequate phenotypic information. While it appears not to be a dominant trait, we cannot rule out a reduced (or "incomplete") penetrance inheritance mode. Also, while Secret Signal Observer from our pedigree is an American Jersey bull, he was born in 1953, and if WF was at high levels in Ewing's 1957 study (21.5%), WF is highly unlikely to have arisen initially from him.

Filtering 1kbulls-Run9 Jersey cattle genotypes found 16,771 variants homozygous for the reference allele in the unaffected half-sibling and heterozygous or homozygous for the alternate allele in all four WF cows (and at <5% of the total 179 Jersey cattle). We observed these variants tended to cluster in regions, most likely the result of high linkage disequilibrium as Australian Jersey cattle are particularly inbred (Scott *et al*. 2021). Interrogating these variants with VEP identified 84 variants within 66 genes as having either a low, moderate or high impact. Most variants were intronic (49%) or intergenic (39%) with no impact assigned. Of the coding variants, 53% were synonymous, 42% were missense, 2% were nonsense, and 2% were frameshift mutations.

The only high impact variant within an annotated gene was a C/A SNP at Chr6:13249536 which creates a stop codon in the *AP1AR* gene (Table 1). AP1AR is involved in negative regulation of receptor recycling and vesicle targeting between the trans-Golgi network and endosomes (Stelzer *et al*. 2016). While there is no known link between *AP1AR* and diseases affecting craniofacial skeletogenesis or maxillofacial dysmorphism, several genes associated with variants classified as having a "moderate" impact do have known associations (Table 1), including *SCARF1*, *BANK1* and *RAB2A* (Rappaport *et al.* 2013). *SCARF1* is expressed in endothelial cells and regulates the uptake of chemically modified low density lipoproteins (Stelzer *et al*. 2016). It has been implicated in Van Den Ende-Gupta syndrome, a congenital autosomal recessive malformation syndrome that effects facial features and the skeletal system in humans (Rappaport *et al.* 2013). *BANK1* is associated in humans with both Parry-Romberg syndrome (facial hemiatrophy), a rare condition involving atrophy of facial components (including the jaw) and Potocki-Shaffer syndrome which effects

craneo bones and facial appearance (Rappaport *et al.* 2013). RAB2A belongs to the Rab family, membrane-bound proteins involved in vesicular fusion and trafficking (Stelzer *et al*. 2016). *RAB2A* has been implicated in cleft palate malformation as well as Warburg Micro syndrome 1, a rare autosomal recessive syndrome effecting facial appearance (Rappaport *et al.* 2013). Interestingly, examining the region around this *RAB2A* variant revealed a large cluster of variants which were heterozygous in the WF cows and homozygous for the reference allele in the unaffected half-sibling.

Most variants were in intronic and intergenic regions (49% and 39% respectively). Regulatory elements (e.g. enhancers) are also located in these regions, therefore these variants should not be completely dismissed. Another unexplored category of variants in this study are structural variants (>50 bp long) that can be more accurately detected using long read sequencing.

Over-representation analysis of VEP genes identified several significantly implicated GO BPs and KEGG pathways (P<0.05). Of particular interest were those genes involved in "endochondral ossification" and "positive regulation of osteoblast differentiation", both essential for bone formation. The ENSBTAG00000037710 gene (unannotated) containing a "high" impact frameshift and *ZNF536* containing two missense SNP with a "moderate" impact were linked to "regulation of transcription, DNA-templated" (Table 1). Also of interest was the KEGG pathway "folate biosynthesis" involving the ENSBTAG00000016748 gene (unannotated) which has two "moderate" impact missense SNP (Table 1). Folate (vitamin B_9) is an essential nutrient long acknowledged as important for foetal growth and development and plays an important role in maintaining bone health.

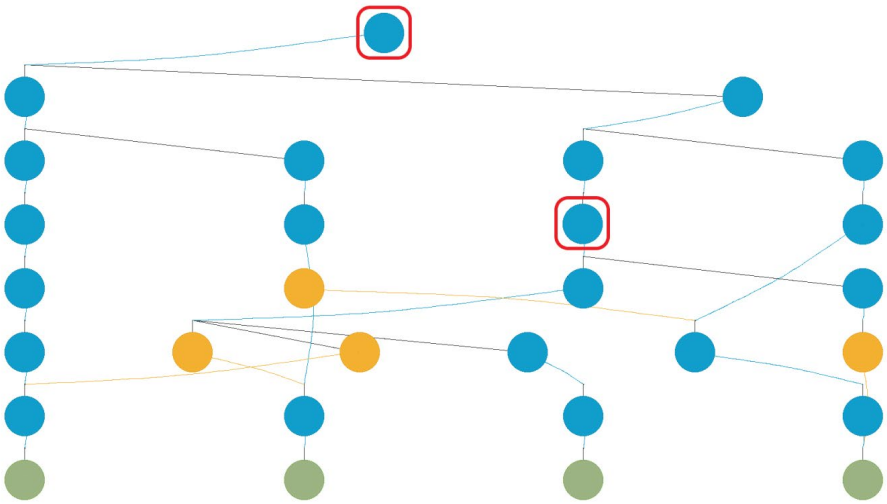

Figure 1. Simplified pedigree for wry face affected cows
Green circles represent the four affected cows, blue circles represent sires and yellow circles represent dams. Common ancestors are highlighted by red boxes. To visually simplify the pedigree, sires and dams not connecting back to common ancestors have been removed.

CONCLUSION

This small study has demonstrated that it may be possible to uncover the genetic cause for WF in the Australian Jersey population. Since multiple putative causal genes have been identified, extra sequencing, including long read sequencing, of affected and unaffected relatives is required to identify the causal mutation(s). This study is being expanded to validate these results and increase the power to detect more potential WF causal variants. Inclusion of causal variants in routine DNA testing may allow farmers to avoid high risk matings that could result in WF offspring.

Table 1. Variants classified as having high or moderate impact

Chr	Position	Variant	Consequence	Impact	Gene	Ensembl ID
6	13249536	C/A	stop codon gained	HIGH	AP1AR	ENSBTAG00000003941
12	71304228	C/T	splice donor	HIGH	-	ENSBTAG00000026070
18	57221530	G/GA	frameshift	HIGH	-	ENSBTAG00000037710
2	117389921	T/A	missense	MODERATE	DNER	ENSBTAG00000016063
2	120041472	C/G	missense	MODERATE	-	ENSBTAG00000016748
2	120041475	C/G	missense	MODERATE	-	ENSBTAG00000016748
3	60257454	C/T	missense	MODERATE	TTLL7	ENSBTAG00000003322
3	76782778	C/T	missense	MODERATE	DEPDC1	ENSBTAG00000001343
4	116914236	G/C	missense	MODERATE	PAXIP1	ENSBTAG00000017505
4	118147886	G/A	missense	MODERATE	RNF32	ENSBTAG00000020335
6	22868432	T/A	missense	MODERATE	BANK1	ENSBTAG00000015297
9	13265164	G/C	missense	MODERATE	CD109	ENSBTAG00000013222
12	72839314	C/A	missense	MODERATE	-	ENSBTAG00000023309
13	23414943	C/T	missense	MODERATE	-	ENSBTAG00000051361
13	27934712	A/G	missense	MODERATE	-	ENSBTAG00000047869
13	42905566	G/A	missense	MODERATE	-	ENSBTAG00000035572
13	43143962	C/T	missense	MODERATE	CALML5	ENSBTAG00000013854
14	8564112	C/T	missense	MODERATE	TMEM71	ENSBTAG00000017138
14	26252117	A/T	missense	MODERATE	RAB2A	ENSBTAG00000000948
14	76196018	T/C	missense	MODERATE	RMDN1	ENSBTAG00000015734
15	6405728	T/G	missense	MODERATE	BIRC3	ENSBTAG00000024918
15	81534709	T/A	missense	MODERATE	OR5B12	ENSBTAG00000049719
18	36479140	G/A	missense	MODERATE	COG8	ENSBTAG00000001665
18	38353565	C/G	missense	MODERATE	ZFHX3	ENSBTAG00000014636
18	41070602	C/T	missense	MODERATE	ZNF536	ENSBTAG00000007262
18	41071302	C/T	missense	MODERATE	ZNF536	ENSBTAG00000007262
18	62698984	C/T	missense	MODERATE	-	ENSBTAG00000049820
18	62921228	C/G	missense	MODERATE	-	ENSBTAG00000050536
18	62972057	G/C	missense	MODERATE	-	ENSBTAG00000038797
18	62972059	A/G	missense	MODERATE	-	ENSBTAG00000038797
19	22747077	G/A	missense	MODERATE	SCARF1	ENSBTAG00000011483
23	13275715	T/C	missense	MODERATE	KIF6	ENSBTAG00000027197
28	35535151	G/T	missense	MODERATE	-	ENSBTAG00000048082
28	35647259	C/T	missense	MODERATE	SFTPA1	ENSBTAG00000023032

REFERENCES

Abdelhakiem M. and Elrashidy M. (2017) *Assiut Vet. Med. J.* **63:** Article 11.

Aiello S.E. and Moses M.A. (2016) 'The Merck Veterinary Manual' 11th ed. Wiley.

Ewing M.B. (1957) Masters Thesis, Kansas State College of Agriculture and Applied Science.

Hayes B.J. and Daetwyler H.D. (2019) *Annu. Rev. Anim. Biosci.* **7:** 89.

Huang da W., Sherman B.T. and Lempicki R.A. (2009) *Nat. Protoc.* **4:** 44.

Luan S. (2018) URL https://github.com/luansheng/visPedigree.

Rappaport N., Nativ N., Stelzer G., Twik M., Guan-Golan Y., Stein T.I., Bahir I., Belinky F., Morrey C.P., Safran M. and Lancet D. (2013) *Database (Oxford)* **2013:** bat018.

Scott B.A., Haile-Mariam M., Cocks B.G. and Pryce J.E. (2021) *J. Dairy Sci.* **104:** 11832. Stelzer G., Rosen N., Plaschkes I., Zimmerman S., Twik M., Fishilevich S., Stein T.I., Nudel R., Lieder I., Mazor Y., Kaplan S., Dahary D., Warshawsky D., Guan-Golan Y., Kohn A., Rappaport N., Safran M. and Lancet D. (2016) *Curr. Protoc. Bioinformatics* **54:** 1.30.1-1..3.