Molecular analysis of White Galloway coat colour variations

Bertram Brenig¹, Claudia Floren¹, Julia Beck², Kirsten Bornemann-Kolatzki², Silvia Henneck¹, Isabel Wiedemann¹, Ekkehard Schütz¹ & Hermann Swalve³

1) Institute of Veterinary Medicine, Georg-August-University Göttingen
2) Chronix Biomedical GmbH, Göttingen
3) Institute for Agricultural and Nutritional Sciences, Martin-Luther-University, Halle/Wittenberg
Coat colour of livestock

• Coat colour variations have always been fascinating for man

• In early mythology and art coat colour of animals played an important role (Siebel, 1997; Forbis, 1980)

• Coat colour variations have been one of the earliest selection criteria (Schmutz, 2002)
Coat colour variations in White Galloway and White Park cattle

- **wsg**: white, well marked
- **wsü**: white, strongly marked
- **wss**: white, mismarked
- **wsch**: white, fully black
Aims of the study

• Elucidation of the genetic background of the different coat colour variations

• Identification of DNA variants associated with the coat colour variations

• Development of a DNA–based test for breeding
Coat colour pathways

Coat colour pathways

(A) E10.5 - 12.5

(Dorsal-lateral migration)

NCCs  MB

(B) E12

(Ventral migration)

NCCs  SCP  MB

Coat colour pathways

Cellular Extension of Melanocyte
Melanin Granules
Golgi Apparatus
Melanocyte Nucleus
Basal Lamina

Epidermis
Demis
Coat colour pathways

Tyrosine

Tyrosine hydroxylase/Tyr

DOPA

DOPA oxidase/Tyr

DOPAchrome

DOPAchrome tautomerase

DOPA quinone

Glutathione or cysteine

Cysteinyldopa

DHICA oxidase/TYRP1

DHICA

Indole-5,6-quinone carboxylic acid

Eumelanin (black form)

Eumelanin (brown form)

DHI oxidase/Tyr

DHI

Indole-5,6-quinone

Alanyl-hydroxybenzothiazine

Pheomelanin (yellow to red)
Coat colour pathways
Coat colour genetics

• Different genes regulate
  – Melanin synthesis
  – Timing of pigmentation
  – Pigment deposition in hairs
  – Pigment distribution in the skin

• Gene interactions modify basic colours

• > 120 genes for coat colours are known in mice
Molecular genetics of bovine coat colours

- 8 genes and corresponding alleles have been described:
  - **KIT** (Mast/stem cell growth factor receptor): Colour-sided, White spotting
  - **KITLG** (KIT ligand): Roan
  - **MC1R** (Melanocortin 1 receptor): Extension
  - **MITF** (Microphthalmia-associated transcription factor): Dominant White, Piebaldism
  - **TYR** (Tyrosinase): Albinism
  - **ASIP** (Agouti signaling protein): Agouti
  - **PMEL** (Premelanosome protein): Dilution
  - **TYRP1** (Tyrosinase-related protein 1): Brown

(Quelle: Durkin et al., 2012, S. Schmutz, 2010, RSH eG)
Identification of causative genes in White Galloway cattle

- Candidate gene analysis $KITLG$, $KIT$, $TYR$ and $MITF$
  - 3 animals of each phenotype
  - Exons, splice-donor/acceptor-sites, UTR
  - Identification of functional SNPs
  - Genotyping of all animals (n=179)

- Genotyping of the $MC1R$ locus
  - $E^D$, $E^+$, $e$
  - n=184
Results of candidate gene-analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Polymorphism</th>
<th>Protein</th>
<th>Genotype</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>KITLG</td>
<td>5' UTR</td>
<td>g.221755T&gt;C</td>
<td>T/T</td>
<td>12</td>
<td>wsch (n=27) 60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T/C</td>
<td>12</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C/C</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5' UTR</td>
<td>g.221761G&gt;A</td>
<td>G/G</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G/A</td>
<td>12</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A/A</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Exon 7</td>
<td>g.45568C&gt;A (roan)</td>
<td>p.A193D</td>
<td>C/C</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TYR</td>
<td>Exon 1</td>
<td>g.66288G&gt;C</td>
<td>p.R255P</td>
<td>G/G</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G/C</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C/C</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>KIT</td>
<td>Exon 5</td>
<td>g.71877602T&gt;C</td>
<td>p.M258T</td>
<td>T/T</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T/C</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C/C</td>
<td>20</td>
<td>88</td>
</tr>
<tr>
<td>MITF</td>
<td>Exon 1</td>
<td>g.136G&gt;A</td>
<td>p.M11</td>
<td>G/G</td>
<td>4*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G/A</td>
<td>9*</td>
<td>6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A/A</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

*KIT: wsü (n=9), wss (n=39); MITF: wsch (n=13), wsg (n=12), wsü (n=9), wss (n=12)
Results of genotyping

• **KITLG, TYR, KIT, and MITF**
  
  – SNPs within the candidate genes do not show association with White Galloway coat colours

• **MC1R**
  
  – 182 black Galloway cattle: e/e, $E^D/E^D$; e/e, $E^D/E^+$
  – 2 red Galloway cattle: e/e, $E^+/E^+$
Whole Genome Sequencing

72.6-73.1 Mb

Log2(ratio)

BTA6: 72013351
BTA6: 72072451
BTA6: 72131551
BTA6: 72190851
BTA6: 72249751
BTA6: 72308851
BTA6: 72367951
BTA6: 72427051
BTA6: 72486151
BTA6: 72545251
BTA6: 72583001
BTA6: 726212101
BTA6: 726870301
BTA6: 726898501
BTA6: 726967801
BTA6: 727234401
BTA6: 727513001
BTA6: 72811201
BTA6: 728939001
BTA6: 729239901
BTA6: 730477801
BTA6: 731066701
BTA6: 73165801
BTA6: 732249901
BTA6: 73343101
BTA6: 73402201
BTA6: 73461301
BTA6: 73520401
BTA6: 73579501
BTA6: 73638601
BTA6: 73697701
BTA6: 73756801
FISH analysis of White Galloway cattle

BAC harbouring *KIT* gene

- Signals on
  - wildtype BTA6 (green)
  - translocation BTA29 (red)
  - BTA3 (yellow)

- Signals on
  - wildtype BTA6 (green)
  - BTA3 (yellow)
**KIT gene translocation**

(Durkin et al., 2012)
Genotype of mismarked White Galloway (wss)

homozygous (Cs_{29}/Cs_{29})
Genotype of fully black White Galloway (wsch)
Genotype of well marked and strongly marked White Galloway (wsg, wsü)
Translocation breakpoint analysis

<table>
<thead>
<tr>
<th>White Galloway Phenotype</th>
<th>n</th>
<th>BTA29 (wt&lt;sub&gt;29&lt;/sub&gt;)</th>
<th>BTA29 (Cs&lt;sub&gt;29&lt;/sub&gt;)</th>
<th>BTA6 (Cs&lt;sub&gt;6&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(total)</td>
<td>αβ&lt;sup&gt;*&lt;/sup&gt;</td>
<td>αD&lt;sup&gt;*&lt;/sup&gt;</td>
<td>EA&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>well marked (wsg)/strongly marked (wsü)</td>
<td>162</td>
<td>162</td>
<td>162</td>
<td>162</td>
</tr>
<tr>
<td>mismarked (wss)</td>
<td>59</td>
<td>0</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>fully black (wsch)</td>
<td>42</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Experimental matings

- 18 matings of White Galloway (wss x wsch)
Conclusions

• Coat colours are inherited in a Mendelian fashion

• Coat colours are caused by a duplication and translocation of the *KIT* gene on chromosome 29 (BTA29, Cs\textsubscript{29})

• Coat colours depend on the number of translocated *KIT* gene copies (dosage effect)

• Development of well marked (wsg) and strongly marked (wsü) phenotypes is still unclear