Analysis of resequencing data suggests that the minipig carries complex disease alleles

C. Reimer[1], C.-J. Rubin[2], S. Weigend[3], K.-H. Waldmann[4], O. Distl[4], H. Simianer[1]

[1] Animal Breeding and Genetics Group, Department of Animal Sciences, Georg-August-University Göttingen, Germany

[2] Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden

[3] Institute of Farm Animal Genetics of the Friedrich-Loeffler-Institut, Neustadt-Mariensee, Germany,

[4] Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Hanover, Germany

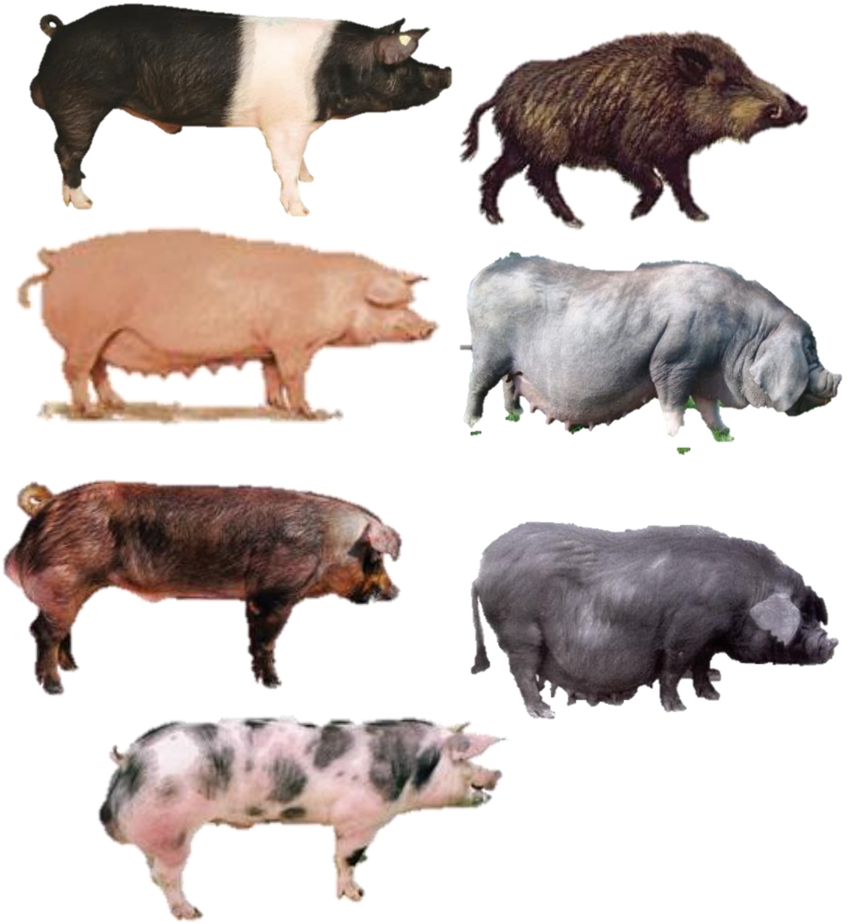
Aims & Content

Is there a genetic link between miniaturization and genetic disorders in Göttingen Minipigs?

1. Genome resequencing and SNP calling
2. Detection of signatures of selection
3. Analysis of salient SNPs and prediction of their effect

Sequences analysed

Public data (Groenen et al., 2012)



A virtual pool of 46 animals of different large pig breeds

vs.

A virtual pool of
11 Goettingen Minipigs
12 Berlin Minipigs

SNP calling from sequences

Alignment with BWA (Li und Durbin, 2009)

Reference genome SusScrofa3 (Archibald et al., 2010)

Sorting with Samtools (Li et al., 2009)

Marking duplicates with Picard-Tools (Picard, 2009)

Discovering variations with GATK (DePristo et al., 2011; McKenna et al., 2010)

Filtering with GATK

➔ 27.6 million SNPs after filtering

Signatures of selection - Methods

Intra-population methods in minipigs

1. z-transformed expected heterozygosity

$$2p(1-p)$$

- $p =$ Allele frequency of the first allele
- 100 kb windows, 80 % overlap
- z-transformation
- Minimum width of 200 kb for a putative sweep

2. Composite likelihood ratio (Nielsen et al., 2005)

- Implemented in software „Sweepfinder“
- Only individuals, subset of 8.5 mio non monomorphic SNPs
- 100 kb windows

Signatures of selection - Methods

Inter-population methods - large vs. small

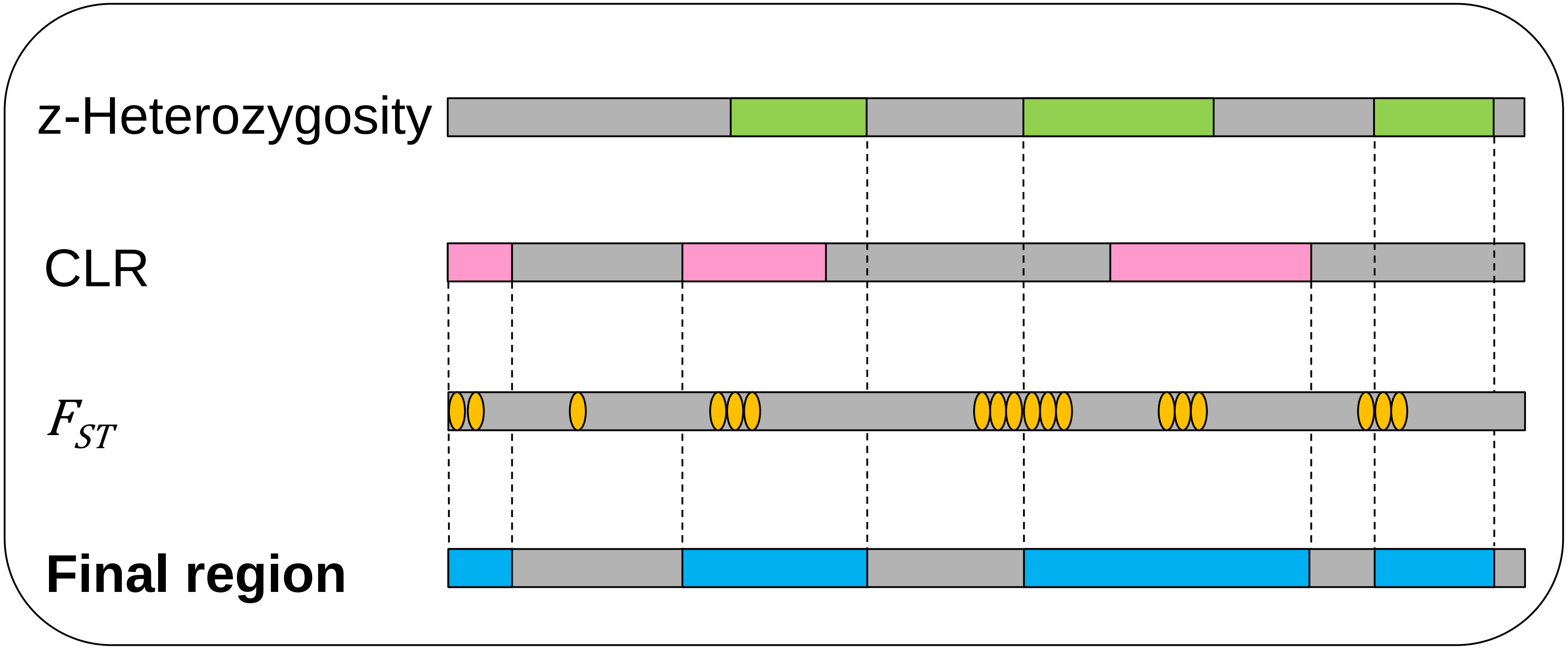
- Fixation Index (F_{ST}) (Weir, 1996, modified)

$$F_{ST} = \frac{\sum n_i (p_i - \bar{p})^2 / 2\bar{n}}{\bar{p}(1 - \bar{p})}$$

  - p_i = frequency of first allele in group i
  - $\bar{p}$ = weighted mean frequency of first allele in both groups
  - n_i = number of samples in group i
  - $\bar{n}$ = average group size
  - Summarized in 100 kb windows with 80 % overlap

Signatures of selection - Methods

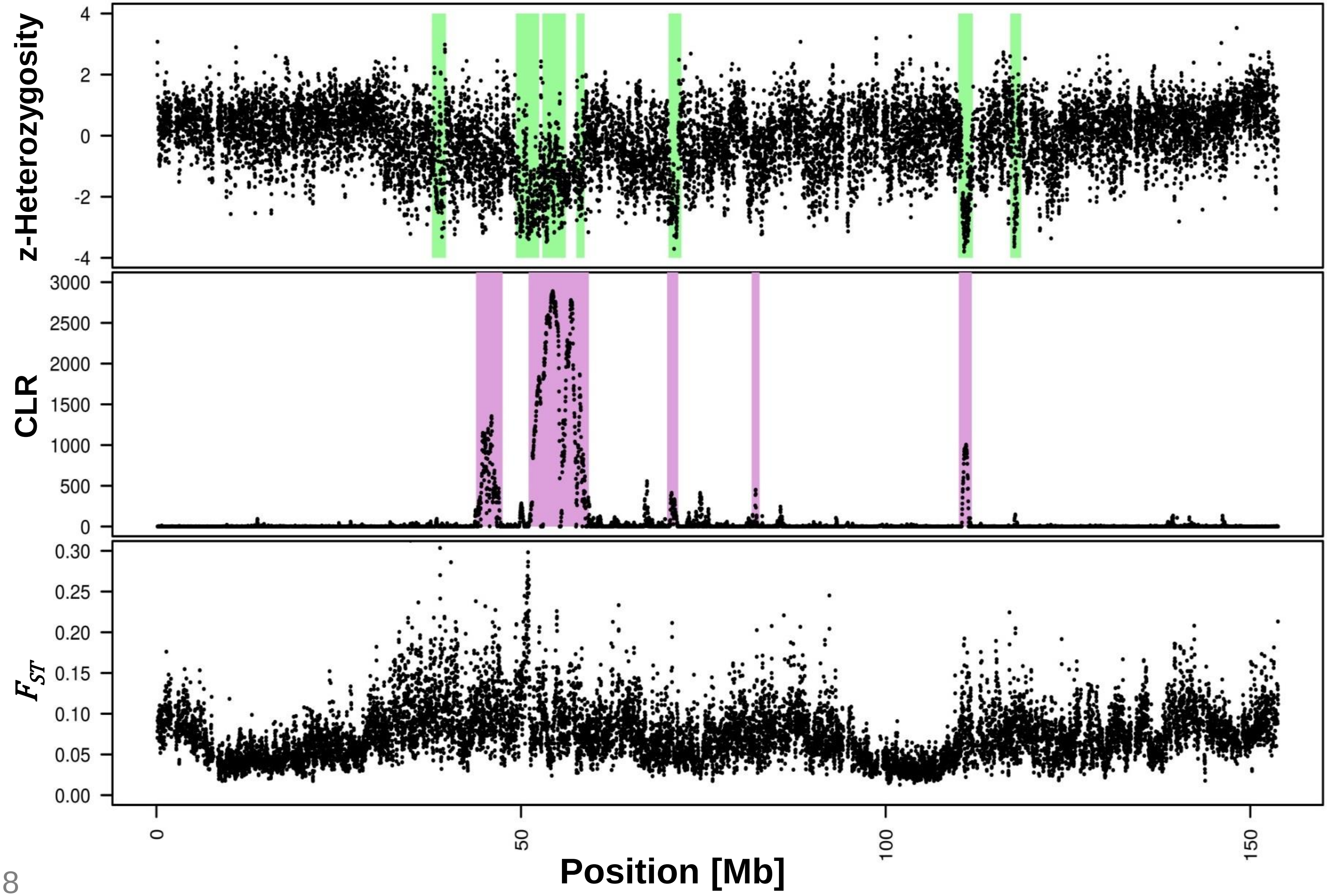
Combination of the three methods to define a final region



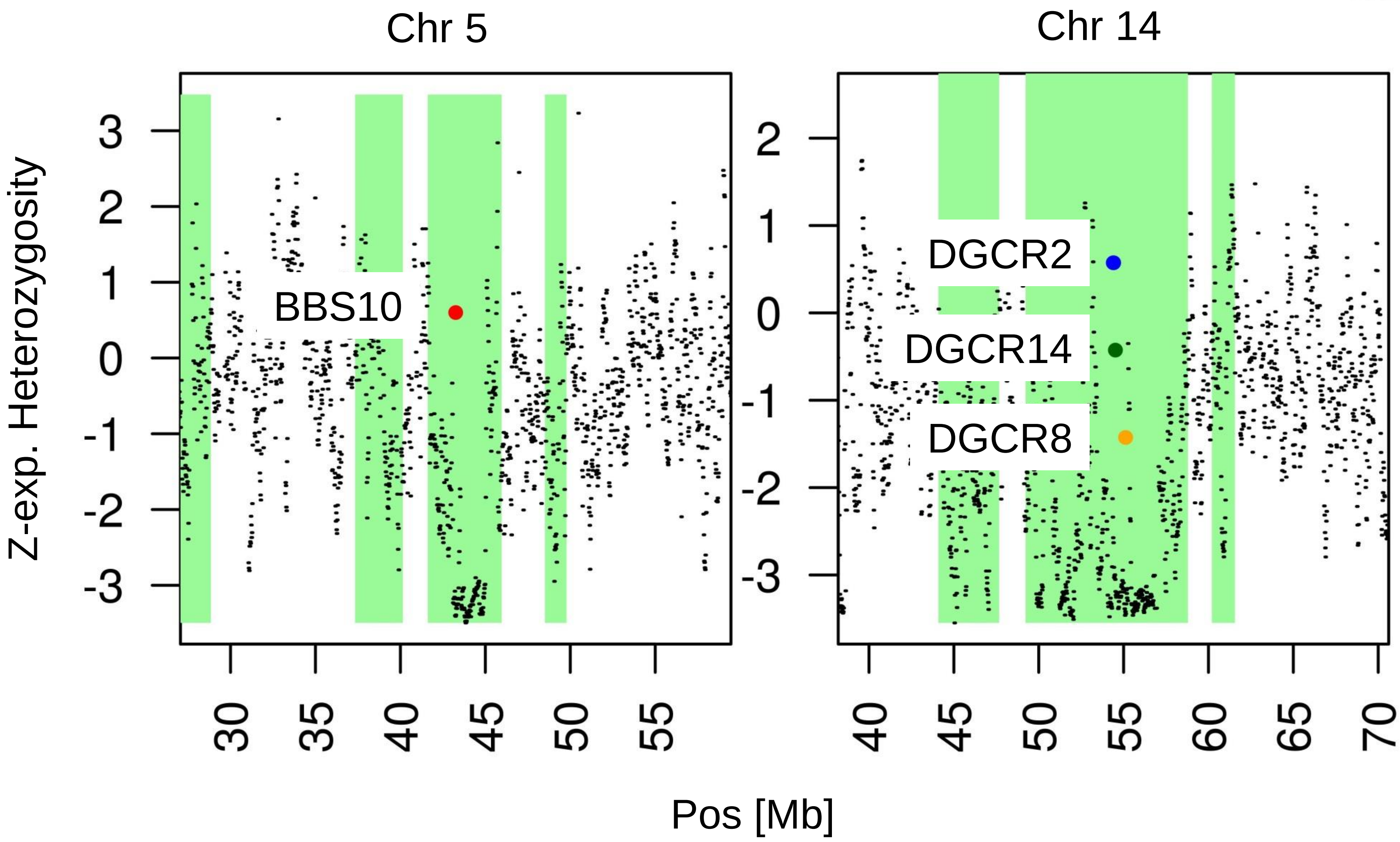
Gene annotation with Ensembl Pig Genes 76 (Flicek et al., 2014)

Signatures of selection - Results

Chromosome 14



Signatures of selection - Results



Complex disease genes

Bardet-Biedl-Syndrome	DiGeorge-Syndrome
Obesity	Seizures
Seizures	Hypocalcemia
Reduced growth	Growth hormone deficiency
Hirschsprungs´ Disease	Skeletal abnormalities
Brachydactyly/ syndactyly	Hypoparathyroidism
Strabismus/ cataracts/ astigmatism	cleft palate
…	…

Related Genes:

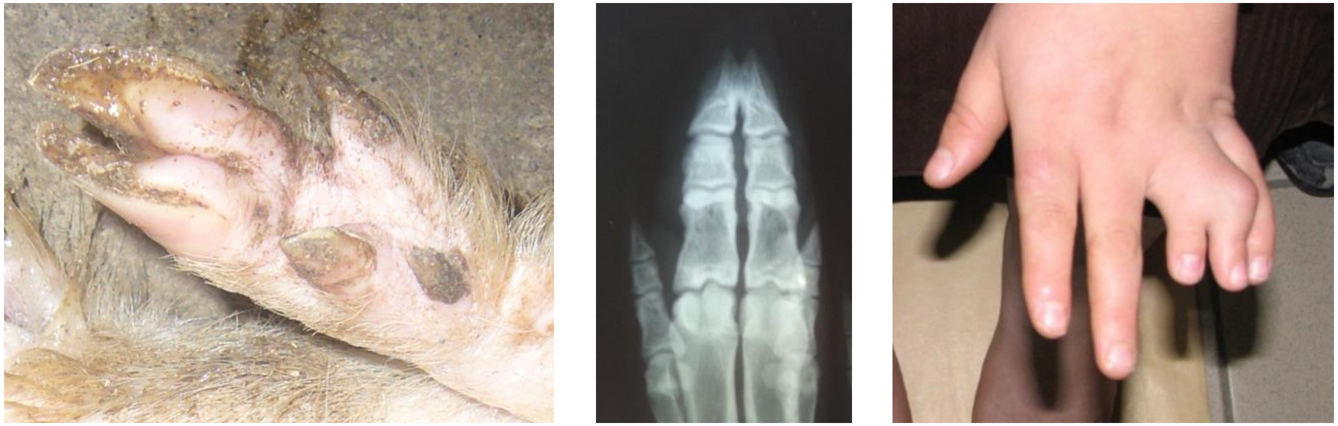
BBS:	BBS2, BBS4, BBS7, BBS10
DiGeorge-Syndrome:	DGCR2, DGCR8, DGCR14

Abnormalities in the Minipigs

Goettingen Minipigs show reduced size and obese phenotypes

Abnormal phenotypes appear at low frequencies

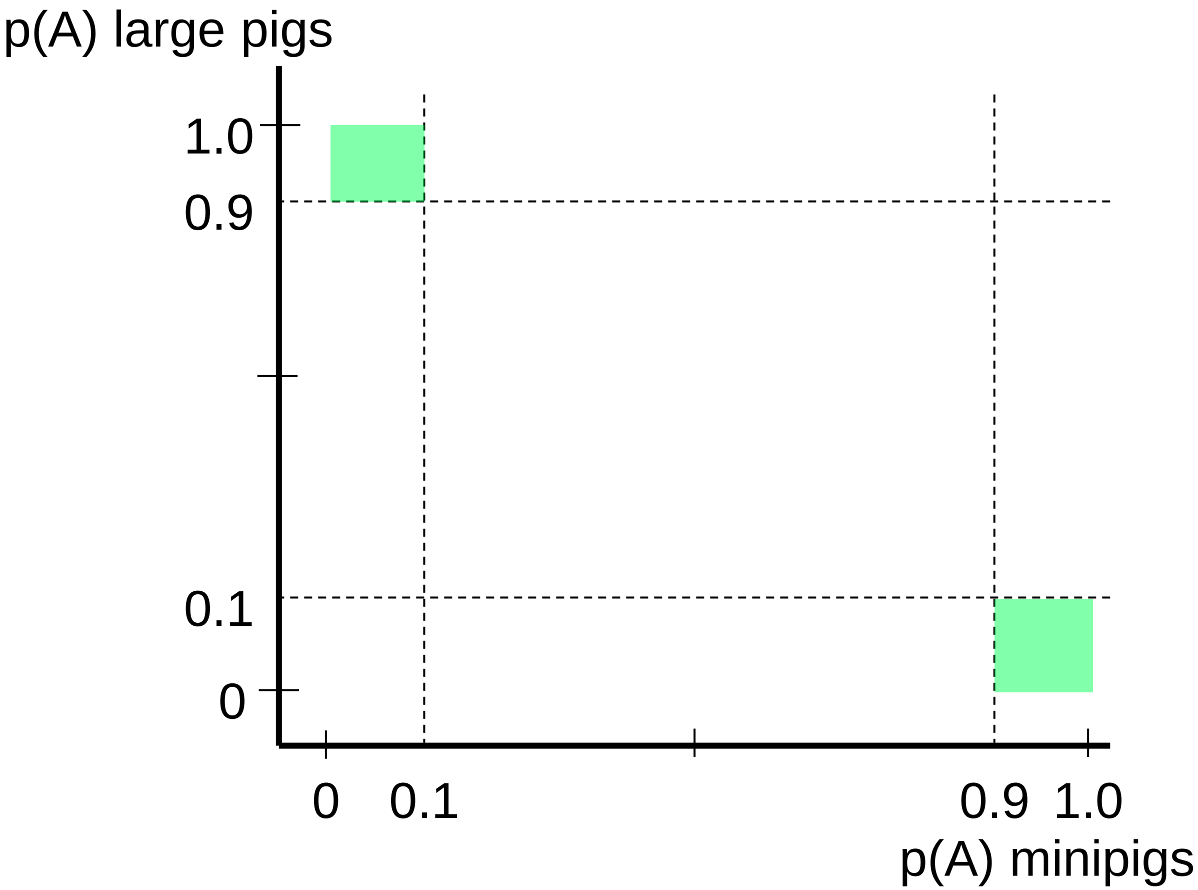
- Seizures (~ 0.8%)
- Brachydactyly/ Syndactyly (~ 2%)
- Strabismus (<< 1%)



Putative Disease Alleles - Methods

Functional Annotation: Variant Effect Predictor (McLaren et al., 2010)

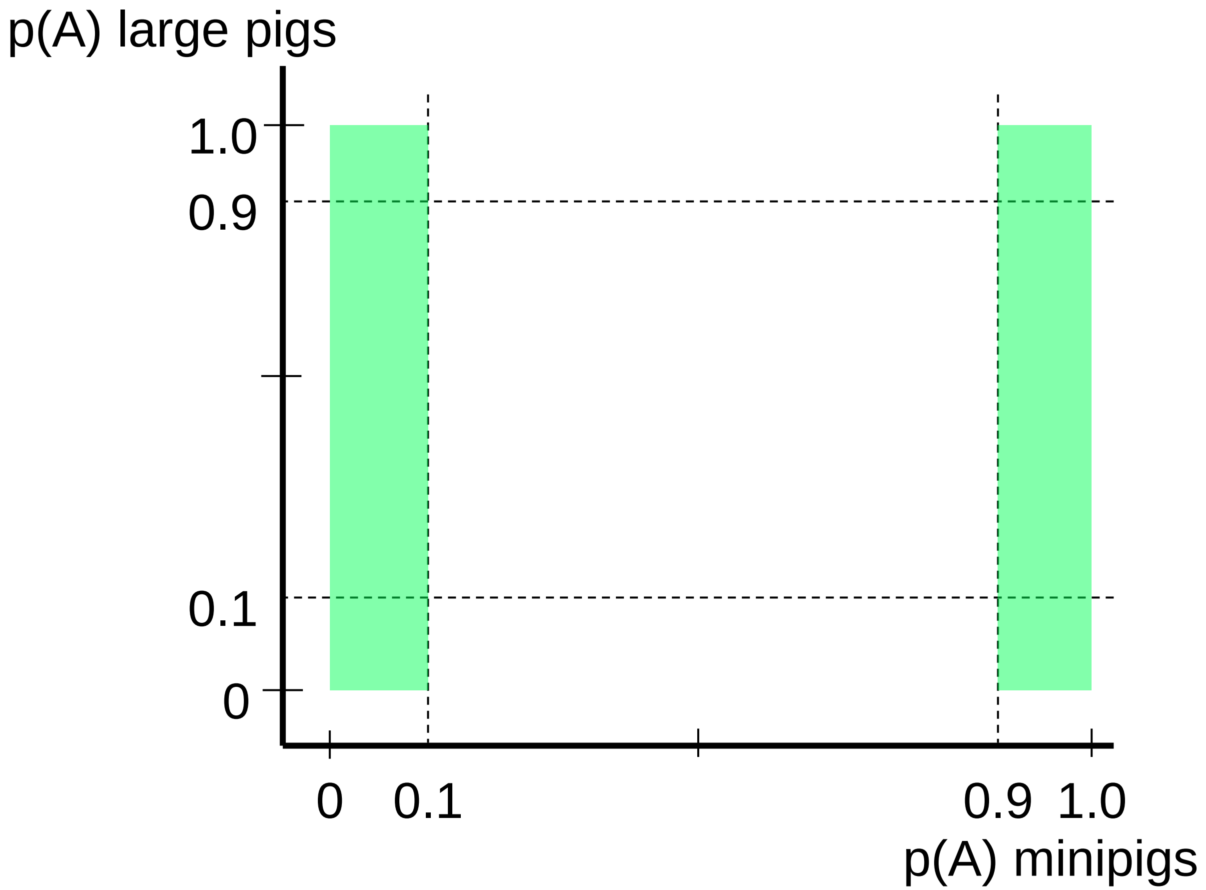
1. Step: All SNPs with an allele frequency difference of 0.8 between large and minipigs



Putative Disease Alleles - Methods

Functional Annotation: Variant Effect Predictor (McLaren et al., 2010)

2. Step: All SNPs extreme allele frequency in Minipigs



VEP – High differences

	3’_UTR	5’_UTR	Initiator_ codon	Intron	missense	Upstream gene
BBS2						
BBS4						
BBS7						1
BBS10						1
DGCR2	6	6		6		3
DGCR8				9		
DGCR14		6		6		
ITGB2		2		13	1	
ssc-mir-1306		6				

Selected genes with reference to BBS, DiGeorge-S. or Hirschsprung

VEP – Extreme frequencies in minipigs

	3'_UTR	5'_UTR	downstream gene	Initiator_codon	Intron	missense	splice_region, Intron	splice_region, synonymous	synonymous	upstream_gene
BBS2	3		2	1	196	7	3	1	7	5
BBS4	4		4		366		1		1	1
BBS7			5		329	1	3		4	6
BBS10						3				1
DGCR2	3	0	4		62					2
DGCR8	12	1	1		45	2			6	2
DGCR14		0	4		9					1
ITGB2	3	1	12		287	1			2	
ssc-mir-1306		0	23							3

Selected genes with reference to BBS, DiGeorge-S. or Hirschsprung

Conclusions

Outstanding sweep signals on chromosome 5 and 14 putatively due to size selection

Bardet-Biedl-Syndrome and DiGeorge-Syndrome genes in these regions

Rare minipig phenotypes resemble symptoms of Bardet-Biedl-Syndrome and DiGeorge-Syndrome

Putatively functional SNP alleles at high frequencies in minipigs

Acknowledgement

Thank you for your attention!

References

Pictures

- http://www.bayerfarm.de
- http://rakgoska.de/
- http://www.lieblingsschwarte.de/
- http://www.britishlivestockgenetics.com
- http://www.mjdrdypu.org
- http://www.ijem.in
- http://www.hermitagegenetics.ie
- http://bartonhillkunes.files.wordpress.com
- http://www.thepigsite.com
- http://www.viarural.com.pe
- http://escholarship.org

Literature

- Archibald et al. (2010) *BMC Genomics*, 11:438.
- DePristo et al. (2011) *Nat Genet,* 43:491-498.
- Flicek et al. (2014) *Nucleic Acids Research,* doi: 10.1093/nar/gkt1196.
- Groenen et al. (2012) *Nature*, doi:10.1038/Nature11622.
- Li und Durbin (2009) *Bioinformatics*, 25, 1754-60.
- Li et al. (2009) *Bioinformatics*, 25, 2078-9.
- McKenna et al. (2010) *Genome Res,* 20:1297-303.
- Nielsen et al. (2005) *Genome Res*, 15:1566-1575.
- Picard (2009) Available: http://picard.sourceforge.net/. Accessed 2013-07-26.
- Weir (1996) *Sinauer Associates, Inc. Publishers*, Sunderland, Massachusetts: ISBN 0-87893-90-4.
- McLaren et al. (2010) *Bioinformatics,* 26:2069-70

Filtering with GATK

- Sequencing depth lower 90 X or higher 840 X
- BaseQualityRS lower -5.5 or higher 5.5
- MappingQualityRS lower -11 or higher11
- ReadPositionRankSum lower -6
- FisherStrand higher 45
- Mapping Quality lower 30
- SNP Clusters with more than 5 SNP within 20 Basepairs

- Individual filtering
  - Genotyping Quality lower 20
  - Depth higher lower 5

Used Samples

Breed	Individual sequence		DNA pool sequence	
Duroc	4	6X		
Hampshire	2	6.5X		
Jiangquhai	1	8.2X		
Large White	14	6.5X		
Landrace	5	6.4X		
Meishan	4	6.8X		
Pietrain	5	5.6X		
European Wildboar	6	6.4X		
Asian Wildboar	5	6.3X		
Göttingen Minipig	11	12.8X		
Berlin Minipig	2	13.9X	1 (10)	13.1X