

In Vivo Selection of a Unique Tandem Repeat Mediated Azole Resistance Mechanism (TR₁₂₀) in *Aspergillus fumigatus* *cyp51A*, Denmark

Appendix

Appendix Table 1. Insight in six non-synonymous SNPs found in the azole resistant isolate P-3 compared to P-1

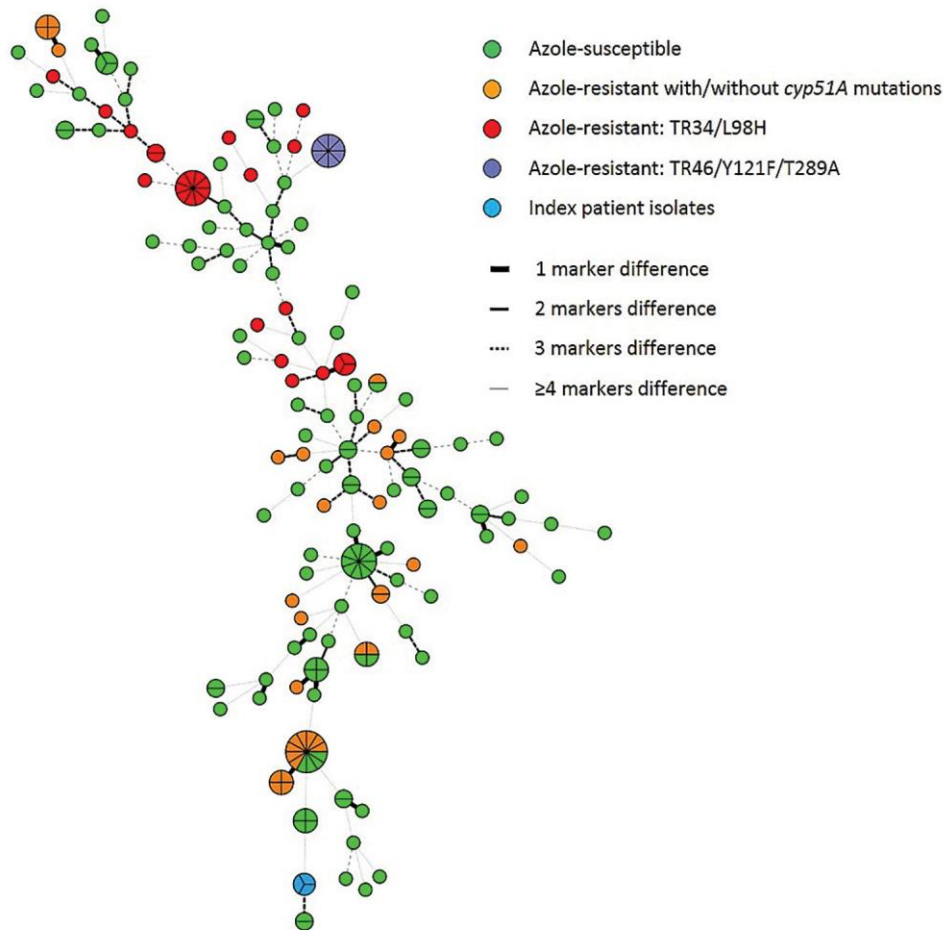
Gene product	Protein ID AF293	Non-synonymous SNP	Amino acid substitution	Comment
Uncharacterized protein. 4-coumarate-CoA ligase	AFUA_1G13110	G1183T	V373*STOP	Precursor of AMP + diphosphate + 4-coumaroyl-CoA→Coumaroyl-coenzyme A is a central intermediate in the biosynthesis of polyketides such as flavonoids (pigmentation) and other natural organic compounds. Mutation leads to a truncated protein from 566 to 373 aa
Uncharacterized protein. Has domain(s) with predicted nucleus localization	AFUA_2G04780	A1873T	D602G	Ortholog in <i>C. albicans</i> is <i>ENP2</i> , encodes an essential protein and a heterozygous mutation confers resistance to 5-fluorocytosine (5-FC) and 5-fluorouracil (5-FU). Amino acid position is not conserved but in a conserved region compared to <i>A. niger</i> and <i>A. nidulans</i> but less for <i>C. albicans</i> (12/15 shared amino acids) and the protein sequences have 70%–82% homology.
<i>FLUG</i>	AFUA_3G07140	C650A	Q216*STOP	Upstream activator of conidiation; required for proper regulation of <i>brlA</i> transcript levels; calcium induced. White phenotype in <i>A. nidulans</i> but not necessarily in <i>A. fumigatus</i> (1). Mutation leads to a truncated protein from 861 to 216 aa. <i>A. fumigatus</i> patient isolate P-3 had a white phenotype.
Uncharacterized protein	AFUA_3G08260	A3192T	K1029M	Orthologs (e.g., <i>smc1</i> in <i>C. albicans</i>). Has cohesin ATPase activity, double-stranded DNA binding, topological DNA entrapment activity, role in mitotic cohesin loading and condensed nuclear chromosome, centromeric region, nuclear mitotic cohesin complex localization. Amino acid position is in a conserved region compared to other <i>Aspergilli</i> (26/31 shared amino acids) and the protein sequences have 84% homology.
Uncharacterized protein. Putative sensor histidine kinase	AFUA_8G06150	G96T	M32I	Ortholog in <i>A. nidulans</i> (AN4815). Amino acid position in non-conserved region compared to <i>A. nidulans</i> and <i>A. oryzae</i> and have 20%–21% protein sequence homology.
Uncharacterized protein. Putative sensor histidine kinase	AFUA_8G06150	C119T	A40V	Same as above. Amino acid position in non-conserved region compared to <i>A. nidulans</i> and <i>A. oryzae</i> and have 20%–21% protein sequence homology.

*Missense mutation, premature stop-codon leading to a truncated gene product.

Appendix Table 2. Insight in 35 SNPs in non-coding/intergenic regions found in the resistant isolate P-3 compared to P-1. The positions listed are in relation to any annotated gene with the closest proximity, upstream or downstream

SNP position in Af293 (Chromosome::position)	SNP	SNP position in relation to annotated gene (AF293)	Protein function	Comment
Chr1::184219	A → G	571 bp upstream, AFUA_1G00580 (PacG/VIB-1)	Putative DNA binding transcription factor	Intergenic/Promoter region
Chr1::184234	A → G	566 bp upstream, AFUA_1G00580 (PacG/VIB-1)	Same as above	Intergenic/Promoter region
Chr1::38596	G → A	1121 bp upstream, AFUA_1G00200	Uncharacterized	Intergenic
Chr1::38605	A → G	1130 bp upstream, AFUA_1G00200	Uncharacterized	Intergenic
Chr1::51920	G → A	2439 bp downstream, AFUA_1G00220	Uncharacterized	Intergenic
Chr1::964093	T → C	717 bp upstream, AFUA_1G03330	Uncharacterized	Intergenic/Promoter region
Chr1::964119	A → G	691 bp upstream, AFUA_1G03330	Uncharacterized	Intergenic/Promoter region
Chr1::964126	C → T	684 bp upstream, AFUA_1G03330	Uncharacterized	Intergenic/Promoter region
Chr1::964130	C → T	680 bp upstream, AFUA_1G03330	Uncharacterized	Intergenic/Promoter region
Chr1::964137	A → G	673 bp upstream, AFUA_1G03330	Uncharacterized	Intergenic/Promoter region
Chr1::964148	A → G	662 bp upstream, AFUA_1G03330	Uncharacterized	Intergenic/Promoter region
Chr1::964154	A → G	656 bp upstream, AFUA_1G03330	Uncharacterized	Intergenic/Promoter region
Chr1::964159	G → C	651 bp upstream, AFUA_1G03330	Uncharacterized	Intergenic/Promoter region
Chr2::4375395	G → T	344 bp downstream of start codon, AFUA_2G16480	Uncharacterized. Has domain(s) with predicted zinc ion binding activity.	Synonymous SNP
Chr3::1025902	C → T	576 bp downstream, AFUA_3G03740	Uncharacterized. Putative protein kinase	Intergenic/Promoter region
Chr3::1126737	G → A	398 bp downstream, AFUA_3G03950	Uncharacterized (<i>ste7</i> in <i>S. cerevisiae</i>)	Intergenic/Promoter region. In Af293, this position is an A. Intron region
Chr3::1445547	G → A	590 bp downstream startcodon, AFUA_3G05900	MAP kinase kinase	Intron region. In Af293, this position is an A.
Chr3::2483009	G → A	419 bp downstream startcodon, AFUA_3G09710	Uncharacterized (<i>uga4</i> in <i>S. cerevisiae</i>)	Intergenic/Promoter region
Chr3::3962289	C → T	177 bp upstream, AFUA_3G15030	Uncharacterized. Has domains with predicted oxidoreductase, zinc binding dehydrogenase family.	Intergenic/Promoter region
Chr3::686389	A → G	7931 bp upstream, AFUA_3G02670	Uncharacterized (<i>lys2</i> in <i>S. cerevisiae</i>). Protein similar to nonribosomal peptide synthases (NRPS-like), encoded in a predicted secondary metabolite gene cluster.	Intergenic region
Chr3::686394	T → C	7926 bp upstream, AFUA_3G02670	Same as above.	Intergenic region
Chr4::1902849	T → C	In intron, 702 bp downstream start codon: AFUA_4G07320	Uncharacterized.	Intron region
Chr5::2055153	C → T	Synonymous SNP: 21 bp downstream start codon: AFUA_5G08120	<i>argJ</i> , Arginine biosynthesis bifunctional protein.	SrbA-regulated during hypoxia.
Chr5::3843678	A → G	163 bp upstream AFUA_5G14865,	Uncharacterized	Intergenic/Promoter region
Chr5::3843680	T → C	161 bp upstream AFUA_5G14865, conserved hypothetical protein	Uncharacterized	Intergenic/Promoter region
Chr5::3843698	G → T	143 bp upstream AFUA_5G14865, conserved hypothetical protein	Uncharacterized	Intergenic/Promoter region
Chr5::3843704	C → T	137 bp upstream AFUA_5G14865, conserved hypothetical protein	Uncharacterized	Intergenic/Promoter region
Chr5::3856240	A → G	1110 bp upstream, AFUA_5G14920	Uncharacterized	Intergenic region
Chr6::2315039	T → C	735 bp upstream, AFUA_6G09500	Uncharacterized (<i>caj1</i> in <i>S. cerevisiae</i>).	Intergenic/Promoter region

SNP position in Af293 (Chromosome::position)	SNP	SNP position in relation to annotated gene (AF293)	Protein function	Comment
Chr6::2508784	T → C	828 bp downstream, AFUA_6G10140	Uncharacterized. Has domain(s) with predicted DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, zinc ion binding activity and role in regulation of transcription, DNA-templated, transcription, DNA-templated.	Intergenic/Promoter region
Chr6::2509191	G → A	1235 bp downstream, AFUA_6G10140	Same as above	Intergenic region
Chr7::1555469	G → A	212 bp downstream, AFUA_7G06350	<i>phoE</i> , Putative phosphate transporter, <i>phoB</i> -regulated	In Af293, this position is an A.
Chr7::1644584	C → G	259 bp downstream, AFUA_7G06770 AND 378 bp upstream, AFUA_7G06760	AFUA_7G06770: Conserved protein of unknown function; protein abundantly expressed in conidia; transcript induced in conidia exposed to neutrophils and to human airway epithelial cells. AFUA_7G06760: Uncharacterized.	Intergenic/promoter region
Chr7::2037815	C → T	5870 bp upstream, AFUA_7G08640	Uncharacterized.	Intergenic region
Chr8::1465035	C → T	G111A→synonymous. Afu8g06150	Uncharacterized. Putative sensor histidine kinase	Synonymous SNP



Appendix Figure. Minimum spanning tree created using BioNumerics (v. 7.6, Applied Maths, Sint-Martens-Latem, Belgium) showing Danish STRAf genotypes with the three index patient isolates colored in light blue. A total of 122 unique genotypes among 191 clinical and environmental isolates, of which 120 genotypes have previously been described (2).

References

1. Mah JH, Yu JH. Upstream and downstream regulation of asexual development in *Aspergillus fumigatus*. Eukaryot Cell. 2006;5:1585–95. [PubMed http://dx.doi.org/10.1128/EC.00192-06](http://dx.doi.org/10.1128/EC.00192-06)
2. Jensen RH, Hagen F, Astvad KMT, Tyron A, Meis JF, Arendrup MC. Azole-resistant *Aspergillus fumigatus* in Denmark: a laboratory-based study on resistance mechanisms and genotypes. Clin Microbiol Infect. 2016;22:570.e1–9. [PubMed http://dx.doi.org/10.1016/j.cmi.2016.04.001](http://dx.doi.org/10.1016/j.cmi.2016.04.001)