Aspergillus fumigatus is the leading cause of fungal infection aspergillosis that kills ~600,000 people annually. This infection is particularly difficult to treat due to the recent emergence of antifungal resistance. A number of mechanisms that make A. fumigatus resistant to theazole class of antifungal have been revealed by applying gene candidate approach. However, less effort has been focused on genes and genetic variants that contribute to fine-scale azole sensitivity.

Genome-Wide Association (GWA) is an unbiased approach that was first applied in humans (Box 1). This approach tests the association between allele frequency and a phenotype of interest. Whole-genome resequencing data can be used for microbial GWA to better understand the genetic contributors of pathogenic phenotypes. In this work, we applied GWA analysis with 69 clinical isolates to identify genetic variants associated with azole sensitivity.

**INTRODUCTION**

**RESULTS**

**BOX 1: Human GWA**

**MATERIAL AND METHOD**

- We generated whole-genome Illumina re-sequencing data for 69 clinical isolates of A. fumigatus from Japan that have paired Minimal Inhibitory Concentrations (MIC) for itraconazole (ITCZ).
- Single nucleotide polymorphisms (SNP) were identified by GATK.
- SNP-based GWA was conducted with PLINK (with PCA correction), PLINK (with permutation testing), and ROADTRIPS. K-mer based GWA was conducted with Pyseer.
- Single gene knockouts (KO) for candidate genes were constructed using CRISPR, and the growth rate of wild-type and KO strains were measured after 72h incubation with and without ITCZ.

**CRISPR in A. fumigatus**

**SNP-based GWA**

**K-mer-based GWA**

**Graphical Abstract**

Figure 1. Overview of microbiome-mediated gene deletion coupled with in vitro assembled dual Cas9 RNP changes. (A) In vivo assembly of dual Cas9 RNP. (B) In vitro assembly of dual Cas9 RNP. (C) Assembly of dual Cas9 RNP. (D) Assembly of dual Cas9 RNP. (E) Assembly of dual Cas9 RNP. (F) Assembly of dual Cas9 RNP. (G) Assembly of dual Cas9 RNP. (H) Assembly of dual Cas9 RNP. (I) Assembly of dual Cas9 RNP. (J) Assembly of dual Cas9 RNP. (K) Assembly of dual Cas9 RNP. (L) Assembly of dual Cas9 RNP. (M) Assembly of dual Cas9 RNP. (N) Assembly of dual Cas9 RNP. (O) Assembly of dual Cas9 RNP. (P) Assembly of dual Cas9 RNP. (Q) Assembly of dual Cas9 RNP. (R) Assembly of dual Cas9 RNP. (S) Assembly of dual Cas9 RNP. (T) Assembly of dual Cas9 RNP. (U) Assembly of dual Cas9 RNP. (V) Assembly of dual Cas9 RNP. (W) Assembly of dual Cas9 RNP. (X) Assembly of dual Cas9 RNP. (Y) Assembly of dual Cas9 RNP. (Z) Assembly of dual Cas9 RNP. (AA) Assembly of dual Cas9 RNP. (AB) Assembly of dual Cas9 RNP. (AC) Assembly of dual Cas9 RNP. (AD) Assembly of dual Cas9 RNP. (AE) Assembly of dual Cas9 RNP. (AF) Assembly of dual Cas9 RNP. (AG) Assembly of dual Cas9 RNP. (AH) Assembly of dual Cas9 RNP. (AI) Assembly of dual Cas9 RNP. (AJ) Assembly of dual Cas9 RNP. (AK) Assembly of dual Cas9 RNP. (AL) Assembly of dual Cas9 RNP. (AM) Assembly of dual Cas9 RNP. (AN) Assembly of dual Cas9 RNP. (AO) Assembly of dual Cas9 RNP. (AP) Assembly of dual Cas9 RNP. (AQ) Assembly of dual Cas9 RNP. (AR) Assembly of dual Cas9 RNP. (AS) Assembly of dual Cas9 RNP. (AT) Assembly of dual Cas9 RNP. (AU) Assembly of dual Cas9 RNP. (AV) Assembly of dual Cas9 RNP. (AW) Assembly of dual Cas9 RNP. (AX) Assembly of dual Cas9 RNP. (AY) Assembly of dual Cas9 RNP. (AZ) Assembly of dual Cas9 RNP. (BA) Assembly of dual Cas9 RNP. (BB) Assembly of dual Cas9 RNP. (BC) Assembly of dual Cas9 RNP. (BD) Assembly of dual Cas9 RNP. (BE) Assembly of dual Cas9 RNP. (BF) Assembly of dual Cas9 RNP. (BG) Assembly of dual Cas9 RNP. (BH) Assembly of dual Cas9 RNP. (BI) Assembly of dual Cas9 RNP. (BJ) Assembly of dual Cas9 RNP. (BK) Assembly of dual Cas9 RNP. (BL) Assembly of dual Cas9 RNP. (BM) Assembly of dual Cas9 RNP. (BN) Assembly of dual Cas9 RNP. (BO) Assembly of dual Cas9 RNP. (BP) Assembly of dual Cas9 RNP. (BQ) Assembly of dual Cas9 RNP. (BR) Assembly of dual Cas9 RNP. (BS) Assembly of dual Cas9 RNP. (BT) Assembly of dual Cas9 RNP. (BU) Assembly of dual Cas9 RNP. (BV) Assembly of dual Cas9 RNP. (BW) Assembly of dual Cas9 RNP. (BX) Assembly of dual Cas9 RNP. (BY) Assembly of dual Cas9 RNP. (BZ) Assembly of dual Cas9 RNP. (CA) Assembly of dual Cas9 RNP. (CB) Assembly of dual Cas9 RNP. (CC) Assembly of dual Cas9 RNP. (CD) Assembly of dual Cas9 RNP. (CE) Assembly of dual Cas9 RNP. (CF) Assembly of dual Cas9 RNP. (CG) Assembly of dual Cas9 RNP. (CH) Assembly of dual Cas9 RNP. (CI) Assembly of dual Cas9 RNP. (CJ) Assembly of dual Cas9 RNP. (CK) Assembly of dual Cas9 RNP. (CL) Assembly of dual Cas9 RNP. (CM) Assembly of dual Cas9 RNP. (CN) Assembly of dual Cas9 RNP. (CO) Assembly of dual Cas9 RNP. (CP) Assembly of dual Cas9 RNP. (CQ) Assembly of dual Cas9 RNP. (CR) Assembly of dual Cas9 RNP. (CS) Assembly of dual Cas9 RNP. (CT) Assembly of dual Cas9 RNP. (CU) Assembly of dual Cas9 RNP. (CV) Assembly of dual Cas9 RNP. (CW) Assembly of dual Cas9 RNP. (CX) Assembly of dual Cas9 RNP. (CY) Assembly of dual Cas9 RNP. (CZ) Assembly of dual Cas9 RNP. (DA) Assembly of dual Cas9 RNP. (DB) Assembly of dual Cas9 RNP. (DC) Assembly of dual Cas9 RNP. (DD) Assembly of dual Cas9 RNP. (DE) Assembly of dual Cas9 RNP. (DF) Assembly of dual Cas9 RNP. (DG) Assembly of dual Cas9 RNP. (DH) Assembly of dual Cas9 RNP. (DI) Assembly of dual Cas9 RNP. (DJ) Assembly of dual Cas9 RNP. (DK) Assembly of dual Cas9 RNP. (DL) Assembly of dual Cas9 RNP. (DM) Assembly of dual Cas9 RNP. (DN) Assembly of dual Cas9 RNP. (DO) Assembly of dual Cas9 RNP. (DP) Assembly of dual Cas9 RNP. (DQ) Assembly of dual Cas9 RNP. (DR) Assembly of dual Cas9 RNP. (DS) Assembly of dual Cas9 RNP. (DT) Assembly of dual Cas9 RNP. (DU) Assembly of dual Cas9 RNP. (DV) Assembly of dual Cas9 RNP. (DW) Assembly of dual Cas9 RNP. (DX) Assembly of dual Cas9 RNP. (DY) Assembly of dual Cas9 RNP. (DZ) Assembly of dual Cas9 RNP. (EA) Assembly of dual Cas9 RNP. (EB) Assembly of dual Cas9 RNP. (EC) Assembly of dual Cas9 RNP. (ED) Assembly of dual Cas9 RNP. (EE) Assembly of dual Cas9 RNP. (EF) Assembly of dual Cas9 RNP. (EG) Assembly of dual Cas9 RNP. (EH) Assembly of dual Cas9 RNP. (EI) Assembly of dual Cas9 RNP. (EJ) Assembly of dual Cas9 RNP. (EK) Assembly of dual Cas9 RNP. (EL) Assembly of dual Cas9 RNP. (EM) Assembly of dual Cas9 RNP. (EN) Assembly of dual Cas9 RNP. (EO) Assembly of dual Cas9 RNP. (EP) Assembly of dual Cas9 RNP. (EQ) Assembly of dual Cas9 RNP. (ER) Assembly of dual Cas9 RNP. (ES) Assembly of dual Cas9 RNP. (ET) Assembly of dual Cas9 RNP. (EU) Assembly of dual Cas9 RNP. (EV) Assembly of dual Cas9 RNP. (EW) Assembly of dual Cas9 RNP. (EX) Assembly of dual Cas9 RNP. (EY) Assembly of dual Cas9 RNP. (EZ) Assembly of dual Cas9 RNP.