

A Polymorphism in *leuS* Confers Reduced Susceptibility to GSK2251052 in a Clinical Isolate of *Staphylococcus aureus*

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GSK2251052 is a broad-spectrum antibacterial inhibitor of leucyl tRNA-synthetase (LeuRS) that has been evaluated in phase II clinical trials. Here, we report the identification of a clinical isolate of *Staphylococcus aureus* that exhibits reduced susceptibility to GSK2251052 without prior exposure to the compound and demonstrate that this phenotype is attributable to a single amino acid polymorphism (P₃₂₉) within the editing domain of LeuRS.

GSK2251052 (GSK'052) is a novel broad-spectrum antibacterial agent that selectively inhibits bacterial leucyl tRNA-synthetase (LeuRS) (1). Although this compound appears to possess many of the requisite properties of an antibacterial drug for treating infection in humans, it also has the undesirable feature of rapidly selecting resistance in bacteria; in phase II clinical trials involving adult subjects suffering from complicated urinary tract infections, resistance to GSK'052 developed within 2 days of administration in three of 14 patients (1, 2). Here, we report that in addition to arising rapidly in bacteria under selection, reduced susceptibility to GSK'052 is preexisting among clinical isolates of *Staphylococcus aureus* that have not been exposed to the drug, a phenomenon that is the result of polymorphism in the drug target.

GSK'052 was obtained by chemical synthesis, according to established methodology (3). To evaluate the susceptibility of staphylococcal strains to this compound, a small panel of *S. aureus* blood culture isolates ($n = 52$) was tested using the microbroth dilution method, according to CLSI guidelines (4). These isolates were recovered from patients at the Erasmus MC University Medical Center Rotterdam (The Netherlands) between November 2009 and May 2010 and therefore originate from a country in which GSK'052 has never been trialed, and during a period that predates the clinical evaluation of GSK'052 (2). Consequently, it may be stated with some confidence that these isolates have never been exposed to this compound in the clinic. GSK'052 exhibited a MIC of 2 to 4 mg/liter against all isolates, with the exception of one (strain 1372), for which the MIC was 16 mg/liter. This degree of reduced susceptibility to GSK'052 is equivalent to that exhibited by a resistant *Escherichia coli* strain selected in a patient upon administration of GSK'052 in the phase II clinical trial, and which was associated with microbiological failure (2).

To determine the mechanism for reduced susceptibility to GSK'052 in *S. aureus* 1372, we proceeded on the basis that this phenotype was likely the result of a polymorphism in the drug target (LeuRS) and subjected the entire *leuS* gene from this strain to PCR amplification and DNA sequence determination. This revealed that in comparison to the *leuS* gene of the fully GSK'052-susceptible laboratory strain *S. aureus* SH1000 (5, 6), *leuS*₁₃₇₂ encodes a protein containing four amino acid polymorphisms (T₃₁₁I, S₃₂₉P, A₅₅₃G, and Y₇₃₅F; the SH1000 residue is shown first in each case).

To establish whether one or more of these polymorphisms ac-

count for the reduced susceptibility to GSK'052, *leuS*₁₃₇₂ and *leuS*_{SH1000} were PCR amplified using oligonucleotide primers ATCGTTATGTCGACTTTTTTATTGAATAGGAGGA and TGCTTAGTGGATCCATTTCAAAGTCCTCCTTAAA (engineered restriction sites shown underlined) and introduced into the staphylococcal expression vector pLOW (7) for ectopic expression in *S. aureus* SH1000. Strain SH1000 (pLOW:*leuS*₁₃₇₂) exhibited a substantial reduction in susceptibility to GSK'052 (MIC, 64 mg/liter) compared to SH1000 (pLOW:*leuS*_{SH1000}) (MIC, 16 mg/liter), confirming that the reduced susceptibility of *S. aureus* 1372 to GSK'052 is indeed a consequence of a polymorphism in LeuRS.

Of the four amino acid polymorphisms in LeuRS₁₃₇₂, two (I₃₁₁ and G₅₅₃) are also found encoded in the *leuS* gene of strains of *S. aureus* whose genome sequences have been deposited in the public databases (strains MRSA252 [GenBank accession no. BX571856] and Mu50 [GenBank accession no. BA000017]). When we tested these strains, neither was more resistant to GSK'052 than SH1000, implying that neither of these polymorphisms participates in reduced susceptibility to GSK'052. Of the remaining two polymorphisms in LeuRS₁₃₇₂, we considered P₃₂₉ the most likely candidate for mediating the relative insensitivity of the enzyme to GSK'052, since it resides within the editing domain, a region of the protein containing the majority of amino acid substitutions identified in the LeuRS of clinical isolates of *E. coli* in which resistance to GSK'052 has evolved (2) (Fig. 1). To test this, the substitution S₃₂₉P was engineered into pLOW:*leuS*_{SH1000} using the Q5 site-directed mutagenesis kit (New England BioLabs, MA, USA) and oligonucleotide primers TTATGTATTACCAACATATGGTACTG (engineered mutation underlined) and TCAGCAATCCAAATTTGTAC. The introduction of this construct into

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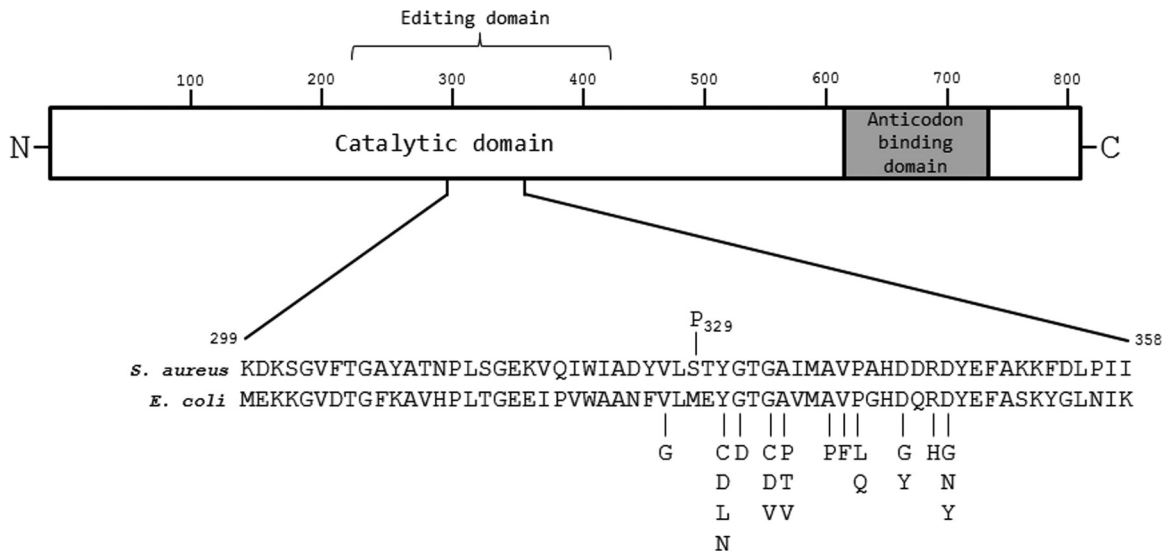


FIG 1 Schematic of the LeuRS protein, with a close up on part of the editing domain, showing the amino acid substitutions that mediate reduced susceptibility to GSK'052 in *S. aureus* (this study) and *E. coli* (2). The residue numbering corresponds to the *S. aureus* sequence, and amino acid substitutions are denoted above and below the sequence alignment for *S. aureus* and *E. coli*, respectively.

SH1000 resulted in a strain exhibiting the same degree of reduced susceptibility to GSK'052 (MIC, 64 mg/liter) as SH1000 (pLOW: *leuS*₁₃₇₂), thereby confirming that the polymorphism P₃₂₉ in LeuRS is responsible for the decreased susceptibility of strain 1372 to GSK'052.

With a view to understanding how this polymorphism negatively impacts the activity of GSK'052 against LeuRS, we examined the published crystal structure of *Thermus thermophilus* LeuRS bound to the parent compound (AN2690) of GSK'052 (PDB identification [ID] 2V0C). AN2690 forms an adduct with tRNA^{leu} that becomes trapped in the editing site of the enzyme (8), with residues lying in close proximity to the P₃₂₉ polymorphism participating in binding the tRNA portion of this adduct. In particular, the preceding residue (L₃₂₉, *T. thermophilus* numbering) forms two hydrogen bonds with nucleotide A76 of the tRNA^{leu} (8). The presence of a conformationally rigid proline adjacent to this position would likely serve to constrain the protein backbone, thereby restricting the conformation of this leucine residue and potentially impairing its ability to make these hydrogen bonds; the loss of one or more hydrogen-bonding contacts would reduce the affinity of the enzyme for the tRNA^{leu} drug adduct and thereby lead to reduced susceptibility to the compound.

In conclusion, we have shown that a polymorphism (P₃₂₉) in the LeuRS enzyme of a clinical isolate of *S. aureus* mediates reduced susceptibility to GSK'052. While our results do not at this stage enable informed speculation regarding the prevalence of or the underlying reason(s) for this polymorphism, it is clear that its presence is in no part attributable to selection by GSK'052. The identification of a clinical *S. aureus* isolate that exhibits uniform reduced susceptibility at the level of the drug target to an experimental antibacterial drug with which it has never been challenged, although a phenomenon that has been reported previously (9), is apparently rare or infrequently documented. Our findings raise the possibility that polymorphisms associated with reduced susceptibility to GSK'052 also exist in strains of other bacterial pathogens and underscore the utility of assessing the activities of anti-

bacterial drug candidates against clinical isolates as part of preclinical evaluation to identify any preexisting mechanisms mediating reduced susceptibility.

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We declare no conflicts of interest.

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