Genetic analysis tools for tuberculosis research

Time-tested and reliable solutions for pathogen detection, host response studies, and drug resistance genotyping

Introduction

Respiratory diseases, such as tuberculosis (TB) [1] and influenza [2], are among the most deadly diseases to have shaped human history [3,4]. TB, caused by the bacterium *Mycobacterium tuberculosis* (MTB), is an ancient disease that has plagued humans for thousands of years. It reached an epidemic state during the 18th and 19th centuries, and continues to be a problem for immunocompromised individuals and in areas with poor health care [15]. The 2020 TB report conducted by the World Health Organization (WHO) identified 30 countries with high TB prevalence and estimated one quarter of the world's population to be infected with MTB [16]. TB is considered one of the top 10 causes of death—with more deaths than from HIV/AIDS—with a death toll of 1.4 million people recorded in 2019 [16]. With TB's continued global presence, multi-drug resistance of MTB is a serious concern and has become recognized as a global crisis and health security threat by the WHO [17]. Continued efforts using genomic analysis tools for detecting MTB, and drug-resistant variants will be of great importance for controlling the spread of deadly MTB. Newly emerging respiratory diseases, such as severe acute respiratory syndrome (SARS) [5,6], Middle Eastern respiratory syndrome (MERS) [7], and the recent COVID-19 (SARS-CoV-2) [8], demonstrate the continuing need for rapid technological advances in disease analysis tools for such pathogens.

Over the last century, analytical methods have rapidly evolved to be on the forefront of accurate and effective infectious disease research and intervention [9,10]. For example, DNA sequence analysis and quantification have become extremely important for the accurate detection of infectious diseases. Techniques such as polymerase chain reaction (PCR) can detect specific sequences, which facilitates the identification of unique pathogens and reveals links between genotype and pathology [11]. Sequencing technologies, such as Sanger sequencing and next-generation

sequencing (NGS) [12], allow researchers to confirm or discover novel changes in DNA sequences. Microarray techniques allow us to analyze tens of thousands of sequences simultaneously, creating genetic profiles that help us understand host-pathogen interactions [13]. Genetic assays allow us to detect the presence of pathogens, determine sequence variants of the pathogen and analyze host-pathogen interactions that are crucial for understanding immune response and susceptibility [14].

Thermo Fisher Scientific has been a leading provider of genetic analysis tools for understanding and monitoring respiratory pathogens. Applied Biosystems™ TaqMan® Assays provide exquisite sensitivity and specificity for detecting unique pathogen genomes and expressed genes. Sanger sequencing using Applied Biosystems™ genetic analyzer capillary electrophoresis (CE) instruments allow researchers to focus on variation and changes in regions of pathogen genomes. Applied Biosystems™ microarrays and Ion Torrent™ NGS systems facilitate discovery-based research that needs little a priori knowledge of specific sequences. Solutions to genetic analysis problems are provided by Thermo Fisher Scientific's toolbox, which continues to improve our understanding of pathogens. This knowledge helps improve human health and mitigate—and hopefully, ultimately prevent—global pandemics.

Pathogen detection

The Thermo Fisher Scientific portfolio of genetic analysis tools, including TaqMan Assays, Sanger sequencing, and Ion AmpliSeq™ technologies, has been used to detect MTB in clinical research samples. Several studies characterized the performance of TaqMan Assays for detecting MTB [18]. These results demonstrated higher sensitivity and reduced turnaround time compared to conventional culture-based methods [19,20]. Specifically, Barletta et al. found 99.5% sensitivity for the true-negative rate in control samples that lack MTB and 94% sensitivity for the true-positive rate for MTB samples in infected groups [21]. Similarly, Onyango et al. found a high sensitivity rate of 96.7% for pathogen detection, including MTB [22]. Additionally, Alcaide et al. found TaqMan Assays successfully identified positive samples that had low mycobacterial loads [19]. Banu et al. assessed Applied Biosystems™ TaqMan® Array cards performance for detecting MTB mutations and found 89% success in detecting wildtype or mutant strains when samples of sputum are smear positive [23]. By the use of TaqMan Assays, researchers have been able to successfully detect and identify MTB [18], determine that host and MTB-encoded circulating miRNAs are potential biomarkers for TB [24], detect *Leishmania* parasites in MTB-positive blood samples [25], and tease apart several respiratory illnesses (e.g., pneumonia, influenza, tuberculosis) [20]. TaqMan Assays have also been useful for identifying MTB in various sample types, including sputum [21], blood [25], cerebrospinal fluid [22], and nasopharyngeal/oropharyngeal swabs [26].

Sanger sequencing has also been useful for identifying the presence of MTB DNA. For example, as part of their study for comparing laboratory techniques, Banu et al. used Sanger sequencing to confirm mutant or wild-type MTB that went undetected by mutant or wild-type probes, respectively [23]. Sanger sequencing was also used to conduct an in-depth analysis of the Rangipo strain of MTB and its association with TB outbreaks in New Zealand [27]. This technique identified three genes in Rangipo isolates linked to TB.

Drug resistance and TB genotyping

The global persistence and number of annual deaths caused by TB has made drug-resistant MTB a top research priority to combat the effects of this disease. It is critical that variants that may be indicators of drug-resistant MTB are identified. To do this, sequence analysis of bacterial genomes has been used to isolate causative mutations. Darban-Sarokhalil et al. found TaqMan allelic discrimination assays successfully detected drug resistance in MTB [28]. Similarly, Banu et al. used TaqMan Assays to detect novel mutations in the *rpoB* gene that are associated with MTB drug resistance to the drugs rifampin and linezolid [23].

Sanger sequencing has also been effective in identifying drug resistance mutations in MTB. For example, Merker et al. successfully identified polymorphisms linked to multi-drug resistance in MTB [29]. Pholwat et al. also used Sanger sequencing to identify 3,200 important basepairs within ten genes associated with TB drug susceptibility [30]. Investigations of resistance to ethionamide, an antibiotic commonly used to treat TB, have also been conducted by Sanger sequencing. Desphande et al. conducted whole-genome and targeted sequencing and identified ethionamide-resistant isolates of MTB by Sanger sequencing [31]. Pyrazinamide is another commonly used antibiotic to treat TB. Using a single-tube PCR assay, Whitfield et al. identified mutations in *pncA* that caused decreased pyrazinamide susceptibility in MTB [32]. Lastly, rifampin has also been used to treat TB; however, Whitfield et al. identified polymorphisms in the *rpoB* locus that were associated with MTB rifampin resistance [33]; the set of alleles may increase susceptibility of MTB to an alternative drug, rifabutin, which opens the potential to optimize treatment for a substantial number of individuals worldwide who are infected with rifampin-resistant MTB.

Host response and host susceptibility

To fully understand tuberculosis pathology, many investigators have researched how MTB affects the biology of infected individuals. Papp et al. investigated changes in RNA expression by using AmpliSeq sequencing of human alveolar macrophages infected with MTB. This study identified important genes that were expressed in response to MTB and suggested that the use of AmpliSeq technology was highly useful in the identification of changes in gene expression associated with respiratory diseases [34]. Chakrabarty et al. investigated MTB biomarkers and used AmpliSeq sequencing to identify microRNA (miRNA) biomarkers in TB-positive individuals. The results revealed the modulation of several miRNAs, including 20 upregulated and 5 downregulated genome-encoded miRNAs, and 6 new genome-encoded microRNAs (MTB-miR1, MTB-miR2, MTB-miR3, MTB-miR4, MTB-miR5, and MTB-miR6), which may have the potential to serve as non-invasive biomarkers for MTB [24].

Several studies have used TaqMan Assays to investigate gene variants and their association with TB [30,35-37]. For example, Sanger sequencing was used to identify a polymorphism in *G6PDH*, which may impair an oxidative burst that predisposes the individual to mycobacterial infections, including TB [38]. Ul Akbar et al. sequenced the *IL12R(β)1* locus in an individual with recurrent TB and found a novel nonsense mutation in the cytokine-binding region [39]. This mutation rendered the cytokine-binding region inactive and thus produced a blunted immune response in the host. Bruiners et al. used Applied Biosystems™ TaqMan® single nucleotide polymorphism (SNP) assays on five SNPs from MTB-positive samples (n=456) and MTB-negative samples (n=448) [35]. Results indicated an association between *C1Q* gene polymorphisms and TB susceptibility. Additionally, using TaqMan Assays, Naidoo et al. found *UGT1A* and *ABCB1* gene variants to increase or decrease pharmacokinetics of moxifloxacin in tuberculosis subjects [37]. Finally, Sadki et al. used TaqMan Genotyping Assays to investigate how polymorphisms in macrophage migration inhibitory factor (*MIF*) and immunoglobulin Fc receptor genes (*FCGR2A* and *FCGR3A*) affect susceptibility to pulmonary tuberculosis (TB) [36]. They found that variants in *MIF*, but not the Fc gene receptors, may affect TB susceptibility.

Another aspect of tuberculosis biology is understanding how the genotypes of infected individuals influence anti-TB drug efficacy for drugs such as isoniazid and rifampin. Naidoo et al. used Applied Biosystems™ TaqMan® Genotyping Assays to examine the pharmacokinetic profiles of rifampin and isoniazid in subjects with drug-susceptible tuberculosis and determined how pharmacogenetic variability in genes coding for relevant drug-metabolizing enzymes affected pharmacokinetic parameters of the two drugs [40]. In another study, Dompreh et al. examined the link between N-acetyltransferase type 2 (*NAT2*) variants and isoniazid pharmacogenetics in Ghanaian children [41]. They found that there may be benefits to screening *NAT2* genotypes when determining effective doses of isoniazid [42].

To better understand host susceptibility to TB, genotypic data should be obtained from populations who are susceptible or resistant to TB. Lu et al. studied the effects of polymorphisms of interferon-gamma (*IFNG*) and its receptor (*IFNGR1*) on TB susceptibility in a Han Chinese population [42]. Using TaqMan technology to perform an allelic discrimination assay to detect the presence or absence of *IFNG* and *IFNGR1* polymorphisms, Lu et al. found that polymorphisms in *IFNG* affected the susceptibility of a Han Chinese individual to tuberculosis. Similarly, Lu et al. investigated the effects of *FOXO3* variants on TB susceptibility and used TaqMan allelic discrimination assays to identify the presence or absence of *FOXO3* variants in suspected TB individuals [43]. They found a polymorphism of *FOXO3* associated with increased risk of TB. Whitfield et al. used Sanger sequencing to assess pyrazinamide susceptibility in subjects with TB and found the assay to be rapid and accurate in predicting pyrazinamide susceptibility [44]. Xu et al. also used Sanger sequencing to assess correlations between polymorphisms in the gene encoding C-reactive protein (*CRP*) and TB susceptibility [45]. They found a correlative increase of TB prevalence in individuals with a *CRP* polymorphism.

Finally, there is interest in developing *in vitro* models for MTB infections. AmpliSeq sequencing was used to investigate pathogenic granuloma formation associated with TB [46] using human peripheral blood mononuclear cells (PBMCs) derived from infected subjects or healthy controls. In this study, the authors suggest that AmpliSeq technology helps in identifying early granuloma formation, contributing to the early detection of TB lung pathology [46].

Conclusion

Respiratory diseases are a serious global health concern, with the World Health Organization (WHO) documenting multiple pandemics and millions of deaths associated with respiratory illnesses[47]. Our ability to detect, quantify, and test variants of disease-causing pathogens to improve treatment and contain outbreaks will be crucial in preventing future pandemics. Thermo Fisher Scientific has been, and continues to be, on the forefront of providing genetic analysis tools for use in infectious disease research. As a trusted partner for genomic research, the genetic analysis tools in the Thermo Fisher Scientific portfolio provide accurate pathogen detection, disease variant and drug resistant identification, and characterization of host-pathogen interactions. TaqMan Assays are extremely sensitive and specific, allowing for strong detection of specific pathogen genomes and expressed genes. Sanger sequencing using Applied Biosystems genetic analyzer capillary electrophoresis (CE) instruments provides researchers with novel opportunities to focus on variation and changes in regions of pathogen genomes. Applied Biosystems microarrays and Ion Torrent NGS systems create discovery-based research that needs little prior knowledge of specific sequences. With continued use of these genomic analysis solutions provided by Thermo Fisher Scientific, some of the world's greatest health scourges may be understood and contained.

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