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Viewpoint

Relevance of genomic diversity of *Mycobacterium tuberculosis* complex in Africa

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ABSTRACT

Background: The diversity in the lineages of *Mycobacterium tuberculosis* complex (MTBC) was initially considered insignificant. However, comparative genomics analysis of MTBC have found genomic variation among the genotypes with potential phenotypic implications.
Objective: Therefore, this viewpoint seeks to discuss the impact of the identified genotypic diversity on the physiology of MTBC and the potential implications on TB control.
Results: Studies conducted in West Africa and other parts of Africa have unravelled the implications of the genomic diversity on phenotypes such as disease outcome, transmission dynamics and host immune response. The understanding of the phenotypic diversity among the different lineages of MTBC may be an important key to the fight against TB.
Conclusion: The relevance of these differences has been observed in the design of new control tools such as diagnostics and anti-TB drugs/vaccines. This only points to the fact that the diversity in MTBC cannot be ignored in future studies especially clinical trials for new vaccines and new anti-TB drugs.

Until the COVID-19 pandemic engulfed the world in early 2020, Tuberculosis (TB) was the top global cause of death from an infectious disease. TB is caused by *Mycobacterium tuberculosis* complex (MTBC) which is made up of a group of closely related acid-fast bacilli that infect specific hosts with occasional cross-species infections. In humans, TB is mainly caused by *M. tuberculosis* sensu stricto (MTBss) and *M. africanum* (Maf) (Gagneux, 2018). The human-adapted species are further classified into seven main phylogenetic lineages (L) with Lineages 1-4 and 7 belonging to MTBss while Lineages 5 and 6 belong to Maf (Gagneux, 2018). Recent studies have identified two additional lineages, L8 and L9, which have been proposed to belong to MTBss and Maf respectively. Interestingly, all these lineages are known to cause disease in Africa, hence making Africa a good geographical setting to study the genotypic and phenotypic diversity of MTBC (Coscolla et al., 2021).

MTBss is globally distributed but Maf is restricted to West Africa for reasons not well understood causing up to 40% of TB cases in some West African countries (Yeboah-Manu et al., 2017). This emphasizes the importance of Maf in West Africa and Africa as a whole. However, Maf has largely been neglected with respect to comparative studies of MTBC. This neglect probably stems from earlier discovery of minimal nucleotide and amino acid diversity of MTBC compared to other bacteria leading to the assumption that the little genotypic diversity of MTBC would not have any significant phenotypic consequence on TB disease and its control. Nevertheless, these discoveries were made using less discriminatory genotyping tools.

Recent studies led by West African institutions have established that there is significant genomic difference between Maf and the other members of MTBC and this diversity have functional implications (Gehre et al., 2013; Otchere et al., 2018). Comparative genomic studies carried out in West Africa have confirmed the distinct genetic variation among the MTBC lineages and even among

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Table 1
Summary of the genotypic and phenotypic diversity of MTBC

	MTBss L4	Maf L5	L6
Genomic diversity			
Nucleotide diversity	Diverse	Conserved	Highly diverse
Selection pressure on human T cell epitopes	Purifying selection	Purifying selection	Positive selection
Selection pressure on regulatory proteins	Purifying selection	Neutral selection	Purifying selection
Mpt64 gene	Intact gene	Mutated gene	Mutated gene promoter
Dominant determinant of INH resistance	*katG* gene mutations	*inhApro* mutations	*inhApro* mutations
Phenotypic diversity			
Growth rate	Grows faster	Grows relatively slower than L4 but faster than L6	Grows relatively slower
Carbon utilization	Effectively utilizes both glycerol and pyruvate	Effectively utilizes pyruvate but sparingly utilizes glycerol	Effectively utilizes pyruvate but sparingly utilizes glycerol
Clinical presentation	Early progression to active disease Relatively good recovery from TB	Delayed progression to disease Poor recovery from TB	Delayed progression to disease Poor recovery from TB
Immune response	Induce relatively lower early inflammatory response	Induce relatively higher inflammatory response	Induce relatively higher inflammatory response
Transmission	Increased transmission	Reduced transmission compared to L4	Reduced transmission compared to L4 and L5
DNA-based diagnostics	Best performance	Poor performance of some new diagnostic kit	Poor performance of some new diagnostic kit
Mpt64-protein-based rapid diagnostics	Very sensitive	Less sensitive	Less sensitive
Current trials and research	The main pathogen for clinical trials	No clinical trial involving Maf L5	No clinical trial involving Maf L6
Basic research on TB bacilli	Globally studied. Majority of TB research involves MTBss	Mostly limited to researchers in West Africa	Mostly limited to researchers in West Africa

the Maf lineages (Table 1) (Gehre et al., 2013; Otchere et al., 2018). Some of the comparative genomic studies have also confirmed genetic diversity even within the sub-lineages of MTBC (Otchere et al., 2018). Will these observed genomic differences among the lineages and sub-lineages have any implication on their phenotypic profiles? These genomic studies have provided insight into the unique biology of Maf showing the need to consider the variation in the phenotypes of the restricted West African lineages compared to the other lineages.

Phenotypic studies have explored the functional implications of these genomic variations including pathogen metabolism, clinical features and transmission dynamics. Castets et al. (1968) observed significant phenotypic diversity between Maf and MTBss using biochemical assays. They reported Maf grow dysgonically on pyruvate-supplemented media and unlike MTBss, Maf showed catalase activity which has been confirmed by several recent studies. Our study also confirmed the diversity in carbohydrate metabolism where Maf showed preference for pyruvate as carbon source compared to glycerol (Osei-Wusu et al., 2021). The preference of L6 for pyruvate as carbon source has been linked to SNPs in the *pykA* and *eno* genes of Maf.

Moreover, it has been shown that MTBss L4 grows faster than Maf and even between the Maf lineages, L5 grows faster than L6 (Table 1) (Osei-Wusu et al., 2021). Comparative phenotypic studies in The Gambia have also reported the slower growth rate of Maf compared to MTBss (Gehre et al., 2013). These findings may suggest that L4 is likely more virulent than the Maf lineages however, studies involving animal models and human derived macrophages are needed to confirm this assertion. This notion may be supported by the discovery that some sub-lineages of the modern lineages such as L4 and L2 may be more pathogenic and transmissible than ancient lineages like Maf lineages which are geographically restricted. The question arises- how this attenuated variant can cause so much disease in West Africa?

Several clinical studies have established diversity in clinical phenotypes of TB caused by different lineages in West Africa. Some strains of MTBC have been associated with large outbreaks while others are considered to be less virulent in specific host populations which confirm the role strain diversity play in pathogenicity. For instance, Maf is associated with reduced transmission compared to MTBss in Ghana (Asare et al., 2018; de Jong et al., 2008). Additionally, while infections by MTBss are associated with early progression to active pulmonary TB after exposure, those by Maf are associated with delayed progression to disease (de Jong et al., 2008). Also, Maf associated TB has been shown to dominate among people living with HIV and/or diabetes which further bolsters the claim that Maf is relatively attenuated compared to MTBss. Furthermore, two studies from West Africa have reported the slower response of Maf infected to anti-TB chemotherapy compared to those infected by L4 (Table 1) (Baya et al., 2020; Diarra et al., 2018). This may suggest a careful study on treatment regimen in Maf endemic areas and the importance of this region in clinical trials. However, all clinical trials are being done outside the West African region.

There is also diversity in host immune responses between individuals infected with Maf and MTBss following standard anti-TB treatment regimen with Maf patients poorly recovering after anti-TB treatment compared to those infected with MTBss (Tientcheu et al., 2016). Moreover, MTBss has been reported to induce lower inflammatory response compared to Maf which could be responsible for the delayed disease progression with Maf (Portevin et al., 2011). Nevertheless, a recent comparative genomics analysis of Maf in Ghana (Otchere et al., 2018) showed the need to consider the two Maf lineages L5 and L6 as different pathogens with potentially different ecological niches. The study identified highly conserved T cell epitopes among L5 which were under purifying selection compared to diverse T cell epitopes among L6 which were under positive selection. This finding suggests that whilst L5 adapts to a definitive host, L6 behaves more like an opportunistic pathogen with wide host-range. Therefore, it is very likely that the immune response to infection caused by these two lineages will differ and will have subsequent consequence on progression of disease. These diversities in immune presentation induced by the different lineages therefore call for further studies to

define where host-directed therapy can be considered as adjunct therapy.

The diversity in the phenotypic and genotypic profiles of MTBC could influence the outcome of TB disease. Interestingly, clinical studies show statistical association between MTBss infection and early progression to active pulmonary TB disease relative to Maf, mostly L6. It may also be that Maf causes less severe symptoms and slower disease progression than MTBss. The differential presentation of infection by different lineages, may be due to numerous genotype-specific mutations in genes that control intermediate metabolism and respiration, cell wall and cell processes, lipid metabolism, regulatory proteins, information pathways and virulence, and adaptation (Yeboah-Manu et al., 2017). The underlying genetic or immunological factors which explain these differences require further study

The observed diversities may have implications on the development of new control tools for TB hence must be considered in the design of these tools. A neglect of the importance of MTBC diversity led to the poor performance of a new diagnostic kit in West Africa (Ofori-Anyinam et al., 2016). This new diagnostic tool, designed based on the MPT64 protein, mostly misdiagnosed L6 as a non-tuberculous mycobacterium (NTM) and showed very low sensitivity for the identification of L6 due to genotypic and phenotypic diversity in the lineages of MTBC (Ofori-Anyinam et al., 2016). This is proof that MTBC diversity cannot be over-looked hence must be factored in the design of control tools.

There are also limited resources committed to research into the understanding of the genotypic and phenotypic diversity of MTBC lineages in Africa. The recent randomized controlled clinical trial that showed the effectiveness of the new 4-month treatment-course for TB did not wholistically consider the restricted lineages of West Africa. There is further limited information on the phenotypic and genotypic diversity of the other African-restricted lineages (L7, L8 & L9) which calls for extensive studies to assess the implication of their diversity in the development of new anti-TB drugs and new TB vaccines.

Transparency declaration

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ethical approval statement

This article did not require ethical approval however, all studies cited in this article had full statements on ethical approval.

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