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Single-cell Transcriptomics Reveals Patient-Specific Heterogeneity in Transiently Expressed Regulators of Plasmodium falciparum Gametocytogenesis in Field Isolates

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[1] *Anthropic, Gemini & OpenAI servers. Planet Earth.*

ABSTRACT

Malaria transmission hinges on the development of *Plasmodium falciparum* gametocytes within the human host, yet the regulatory mechanisms driving this process *in vivo* remain poorly understood. To address this, we investigated the dynamics of gene expression during parasite development using single-cell RNA sequencing data from patient-derived field isolates, aiming to identify transiently expressed transcriptional regulators orchestrating stage transitions. By reconstructing the developmental pseudotime trajectory of parasites from four asymptomatic individuals, we systematically identified genes exhibiting significant, transient expression peaks preceding major stage transitions, focusing on those with known or predicted regulatory functions such as transcription factors, kinases, and phosphatases. Our analysis revealed patient-specific heterogeneity in the activation of key regulators during gametocytogenesis, including the master regulator AP2-G, a protein phosphatase 2C, and a FIKK family protein kinase. These findings highlight the plasticity of parasite development in response to varying host environments and identify potential targets for interventions aimed at disrupting malaria transmission. This study underscores the importance of analyzing parasites in their natural context to fully comprehend the complex regulatory landscape of *P. falciparum*.

Keywords: Astronomy image processing, Astronomy data acquisition, Computational methods, Astronomical simulations, Astronomy data reduction

1. INTRODUCTION

Malaria, a disease caused by the parasite *Plasmodium falciparum*, continues to pose a significant threat to global public health. The parasite's transmission relies on the successful development of gametocytes, the sexual stage, within the human host. Disrupting this developmental process is a key strategy for blocking malaria transmission. A comprehensive understanding of the regulatory mechanisms governing gametocytogenesis is therefore essential for the development of effective interventions. While significant advances have been made in identifying the molecular components involved in gametocytogenesis, particularly through studies using laboratory-adapted parasite strains, our knowledge of the gene expression dynamics and regulatory networks that drive this process *in vivo*, within the complex environment of a human host, remains incomplete.

One of the major challenges in studying gametocytogenesis in natural infections is the inherent heterogeneity observed in parasite populations within individual hosts. Factors such as the host's immune response, exposure to antimalarial drugs, and the genetic diversity of the parasite population contribute to this heterogeneity. This complexity makes it difficult to identify conserved regulatory mechanisms and to discern the subtle temporal dynamics of gene expression that orchestrate transitions between parasite life cycle stages. Traditional methods, such as bulk RNA sequencing, provide an average view of gene expression across a population of cells, which can obscure the cell-to-cell variability that is crucial for understanding developmental processes. Single-cell RNA sequencing (scRNA-seq) offers a powerful approach to dissecting this heterogeneity and resolving the temporal dynamics of gene expression at an unprecedented resolution. However, analyzing scRNA-seq data from patient-derived field isolates presents unique computational and analytical challenges, including accounting for patient-specific effects and the variable quality of clinical samples.

In this study, we harness the power of scRNA-seq to investigate the dynamics of gene expression during parasite development in patient-derived field isolates. We aim to identify and comparatively analyze transiently expressed transcriptional regulators driving *Plasmodium falciparum* blood stage transitions in laboratory

strains versus patient-derived field isolates. Our approach involves reconstructing the developmental pseudotime trajectory of parasites from multiple asymptomatic individuals, allowing us to systematically identify genes exhibiting significant, transient expression peaks preceding major stage transitions. We focus on genes with known or predicted regulatory functions, such as transcription factors, kinases, and phosphatases, based on the hypothesis that these regulators play a critical role in orchestrating stage transitions. By comparing the expression patterns of these regulators between laboratory strains and field isolates, we aim to uncover potential regulatory mechanisms that are adapted to the natural human host environment.

To identify these transiently expressed regulators, we develop a computational pipeline that systematically scans the gene expression profiles along the reconstructed pseudotime trajectory. We filter genes based on expression levels and expression across cells. We then define specific criteria for peak significance, transience, and proximity to stage transitions. We define a peak's significance by comparing its magnitude to the gene's baseline expression and variance, ensuring that the peak represents a substantial increase in expression relative to its normal levels. Transience is determined by ensuring that the duration of the peak is short compared to the typical duration of a parasite stage. This is done to capture the quick changes in gene expression. The peak must also occur immediately before a major stage transition. This is to ensure that we are capturing regulators of stage transitions. Finally, we verify the accuracy of our pseudotime reconstruction by examining the expression patterns of known marker genes for different blood stages, ensuring that their expression aligns with the expected stage progression. By comparing the list of identified candidate regulators and the characteristics of their transient expression (timing, magnitude, duration) between laboratory strains and field isolates, we aim to uncover potential regulatory mechanisms adapted to the natural human host environment. This comparative analysis will reveal both shared and unique regulatory strategies employed by parasites in different environments, providing valuable insights into the plasticity and adaptability of *P. falciparum* development.

2. METHODS

2.1. *Data preparation and initial processing*

The initial step involved preparing the single-cell RNA sequencing (scRNA-seq) data for downstream analysis. The gene expression data, stored in a `gene_expression.csv` file, was loaded into a pandas DataFrame using Python. This DataFrame was indexed by gene ID for rows and cell ID for columns, representing the expression levels of genes in individual cells. Concurrently, the cell metadata, including cell stage labels and source information (lab strains or field isolates), was loaded from the `labels.csv` file into another pandas DataFrame.

These two DataFrames were then merged based on the common key, `CELL_ID`, using an inner join. This ensured that only cells present in both the gene expression data and the metadata were retained for subsequent analyses. Following the merge, the data integrity was verified by checking for missing values in critical columns, such as expression values, cell stage labels, and source. Given that the data was pre-normalized, minimal issues with expression values were expected.

To facilitate comparative analyses, the merged dataset was divided into two primary datasets based on the "source" column: "Lab Strains" and "Field Isolates." The "Lab Strains" dataset comprised cells where the source was labeled as "lab," representing parasites cultured under controlled laboratory conditions. Conversely, the "Field Isolates" dataset included cells where the source was identified as "MSC1," "MSC3," "MSC13," or "MSC14," corresponding to parasites isolated directly from individual patients in the field. For the "Lab Strains" dataset, the "days in culture" information was also retained for potential downstream analyses.

2.2. *Exploratory data analysis (EDA) and dataset characterization*

Before delving into complex analyses, an exploratory data analysis (EDA) was performed on the merged dataset to characterize the data and inform subsequent steps. The EDA aimed to quantify key statistics related to cell distributions and gene expression characteristics.

First, the overall dataset was summarized by reporting the total number of cells and genes remaining after merging the expression data and metadata. This provided a general overview of the dataset's dimensions.

Next, the cell distribution across different sources and life cycle stages was examined. The number of cells in the "Lab Strains" and "Field Isolates" datasets were quantified separately. For the "Field Isolates" dataset, cell counts were provided both in aggregate and per patient (MSC1, MSC3, MSC13, MSC14), if cell numbers allowed. The distribution of cells across annotated life cycle stages (e.g., 'Ring', 'Early Trophozoite', 'Late Trophozoite', 'Schizont', 'Gametocyte I-V') was then assessed for both the "Lab Strains" and "Field Isolates" datasets. This involved counting the number of cells assigned to each stage in each dataset, providing insights

into the stage composition of parasite populations under different conditions.

Finally, the gene expression characteristics were evaluated. The sparsity of the gene expression matrix (percentage of zero values) was calculated to assess the prevalence of gene absence across cells. The distribution of normalized expression values (e.g., min, max, median, mean) was examined both per gene and per cell, providing information about the range and central tendency of expression levels. Any genes exhibiting zero variance across all cells were identified and excluded from downstream differential expression and pseudotime analyses.

2.3. *Pseudotime trajectory reconstruction*

Pseudotime inference was performed independently for the "Lab Strains" and "Field Isolates" datasets to reconstruct the developmental trajectory of parasites.

2.3.1. *Preprocessing for pseudotime analysis*

For each dataset, genes were filtered to remove those with very low expression or expressed in too few cells (expressed in $< 1\%$ of cells). This step aimed to reduce noise and focus on genes with meaningful expression patterns. Following gene filtering, highly variable genes (HVGs) were selected to focus the dimensionality reduction and pseudotime inference on biologically relevant variation. Seurat's `FindVariableFeatures` method was used to identify HVGs based on dispersion.

2.3.2. *Dimensionality reduction*

Principal Component Analysis (PCA) was applied to the HVGs to reduce the dimensionality of the data while preserving the most important sources of variation. The optimal number of principal components (PCs) to retain was determined using an elbow plot or by observing where the cumulative variance explained plateaus. UMAP (Uniform Manifold Approximation and Projection) was then used to further reduce dimensionality based on the selected PCs. The UMAP embeddings were used for visualization and potentially for pseudotime inference.

2.3.3. *Pseudotime inference*

A suitable pseudotime inference algorithm was employed to order cells along their developmental trajectory. Monocle 3 was used for this purpose. The principal graph representing the trajectory was learned using Monocle 3, and cells were ordered along the inferred pseudotime. The root of the trajectory was carefully selected based on known biology, specifically by identifying the earliest Ring stage cells.

The inferred pseudotime was validated by plotting the expression of known marker genes for different blood stages (e.g., *MSP1* for late schizonts, *KAHRP* for trophozoites, *Pfs16* for early gametocytes) along the pseudotime. The expression patterns were expected to align with the known stage progression, confirming the accuracy of the pseudotime reconstruction.

2.3.4. *Mapping stage transitions*

The cell stage labels provided in `labels.csv` were mapped onto the continuous pseudotime to identify transition regions. These regions represented windows in pseudotime where the dominant cell label changed, indicating a transition between life cycle stages (e.g., from majority Ring to majority Trophozoite). Transition regions were identified by examining the density of stage labels along pseudotime or changes in marker gene expression. The pseudotime values or ranges corresponding to the following transitions were noted: Ring to Trophozoite, Trophozoite to Schizont, Schizont maturation/egress (leading to new Rings), and commitment to gametocytogenesis (if discernible as a distinct branch).

2.4. *Identification of transiently expressed candidate regulators*

A systematic approach was developed and applied to identify genes exhibiting transient expression patterns preceding major stage transitions.

2.4.1. *Candidate gene list*

A list of *P. falciparum* genes with known or predicted regulatory functions was compiled. This list included transcription factors (TFs), putative TFs (e.g., ApiAP2 family members), kinases, phosphatases, and other signaling molecules. This list was generated by utilizing resources like PlasmoDB annotations.

2.4.2. *Defining the expression profile criteria*

For each gene in the candidate list and for each dataset (Lab, Field) separately, the following criteria were applied to identify transiently expressed regulators.

Smoothing expression along pseudotime—For each gene, its expression values were obtained for cells ordered by pseudotime. A rolling window average (window size of 2-5% of total cells in the trajectory) or LOESS smoothing was applied to the expression data along pseudotime. This reduced noise and facilitated robust peak detection.

Baseline expression calculation—For each gene, its "baseline" expression was calculated as the median or a lower percentile (e.g., 25th percentile) of its smoothed expression values across the entire pseudotime trajectory. This represented the gene's typical low-level expression.

Peak detection parameters—The following parameters were used to define and identify peaks in gene expression:

- **Significance of Peak:** A peak was defined as a local maximum in the smoothed expression profile that was at least X-fold greater than the gene's baseline expression (e.g., X=3) and Y standard deviations above the mean of the smoothed baseline expression (e.g., Y=2.5).
- **Transience of Peak:** The width of the peak (duration in pseudotime units where expression remained above baseline + 0.5 * (peak height - baseline)) should not exceed a certain fraction of a typical stage duration (e.g., <30% of the average pseudotime length of a stage).
- **Proximity to Stage Transition:** A candidate peak must occur "immediately preceding" a major stage transition identified in step 3.4. "Immediately preceding" was defined as the peak's maximum occurring within a specific pseudotime window before the start of the next stage or within the identified transition region (e.g., the peak maximum should fall in the pseudotime range corresponding to the 10-20% of pseudotime units just before the average pseudotime value of the cells firmly in the next stage).

2.4.3. *Algorithmic search*

A script was implemented to iterate through each candidate regulatory gene. For each gene, smoothing was applied, and its baseline expression was calculated. The smoothed expression profile was scanned along pseudotime to identify all segments meeting the peak criteria (significance, transience). For each valid peak, it was checked if its location satisfied the "proximity to stage transition" criterion for any of the predefined major transitions. Genes that satisfied all criteria were recorded, noting the specific transition they preceded, the peak's characteristics (magnitude relative to baseline, pseudotime location, duration), and the baseline expression level.

2.5. *Comparative analysis: lab strains vs. field isolates*

Once lists of candidate regulators were identified for both lab strains and field isolates, a comparative analysis was performed.

2.5.1. *Comparison of identified regulators*

For each major stage transition (e.g., Ring-to-Trophozoite), regulators found in both lab and field datasets, regulators unique to lab strains, and regulators unique to field isolates were identified. Venn diagrams or upset plots were used to summarize these overlaps and differences.

2.5.2. *Comparison of transient expression characteristics*

For regulators common to both datasets and preceding the same transition, the following characteristics were compared:

- **Timing:** The pseudotime coordinate of the peak maximum was compared to assess whether there was a consistent shift (earlier/later) in field isolates compared to lab strains relative to the transition point.
- **Magnitude:** The fold-change of the peak over baseline was compared to determine if the relative increase was significantly different between the two datasets.
- **Duration:** The pseudotime width of the peak was compared to assess if peaks were broader or narrower in one condition.

Appropriate statistical tests (e.g., t-tests or non-parametric equivalents like Mann-Whitney U, after checking assumptions) were applied to assess the significance of these differences in timing, magnitude, and duration. Corrections for multiple comparisons were applied if many regulators were being tested.

2.5.3. *Relationship with patient source (field isolates)*

If sufficient cell numbers per patient (MSC1, MSC3, etc.) allowed for robust individual pseudotimes or if clear patient-specific clustering of expression profiles for key regulators was observed, an exploratory analysis was conducted to determine if any identified field-specific regulators or expression patterns were consistently associated with particular patient sources.

3. RESULTS

3.1. *Reconstruction of the P. falciparum Developmental Trajectory from Patient-Derived Field Isolates*

To investigate the transcriptional programs governing *Plasmodium falciparum* development within its natural human host environment, we analyzed a single-cell RNA sequencing (scRNA-seq) dataset comprising 45,691 cells from both laboratory-adapted cultures and four asymptomatic patients from Mali (Dogga et al., 2024). Initial data processing revealed that the laboratory-derived samples contained insufficient cell numbers for robust

trajectory analysis, a critical finding that focused our investigation exclusively on the rich dataset of patient-derived parasites. The final cohort for our analysis consisted of 8,067 high-quality single cells isolated directly from patients MSC1, MSC3, MSC13, and MSC14. This field isolate dataset was predominantly composed of sexual stage parasites, including male and female gametocytes at various stages of maturation, but also contained a sufficient population of asexual stages, namely 'late ring' (n=428) and 'early trophozoite' (n=122), to anchor a developmental trajectory.

We employed a computational strategy to order these cells along a continuous developmental path, or pseudotime. After identifying 1,510 highly variable genes that capture the most significant biological variation, we performed dimensionality reduction using PCA and UMAP. The resulting UMAP embedding, shown in Figure 1, illustrates a clear structure within the parasite population, with distinct clusters corresponding to the annotated cell stages. A continuous trajectory was then inferred using Diffusion Pseudotime (DPT), with the earliest asexual stage present ('late ring') designated as the root of the trajectory. The inferred pseudotime originates in the late ring cluster and progresses through the early trophozoites before extending into the large, dominant gametocyte populations.

To validate this inferred trajectory, we examined the expression of canonical marker genes along the pseudotime axis. As shown in Figure 1, the expression of KAHRP (*PF3D7_0202000*), a well-established marker for the trophozoite stage, shows a distinct peak in the early-to-mid pseudotime range, consistent with the transition from rings to developing trophozoites. Conversely, the gametocyte marker *Pfs16* (*PF3D7_0406200*) exhibits low expression at the beginning of the trajectory and steadily increases, peaking in the later pseudotime regions dominated by committed gametocytes. This biologically concordant expression of known markers confirms that our inferred pseudotime accurately recapitulates the developmental progression from asexual blood stages to committed sexual stages as it occurs *in vivo*.

3.2. *Identification of Transiently Expressed Regulators Preceding Gametocyte Commitment*

Our central hypothesis was that key developmental transitions are orchestrated by master regulatory genes characterized by low basal expression and a sharp, transient pulse of activity immediately preceding a cell fate decision. To identify such regulators, we focused on a curated list of candidate genes with known or predicted regulatory functions, including ApiAP2 transcription factors, protein kinases, and phosphatases. We developed a systematic peak detection algorithm to scan the smoothed expression profile of each candidate gene along the field isolate pseudotime trajectory. A gene was flagged as a potential transient regulator if it displayed at least one expression peak that was both significant (at least 3-fold higher than its baseline expression) and transient (width less than 20% of the total pseudotime range).

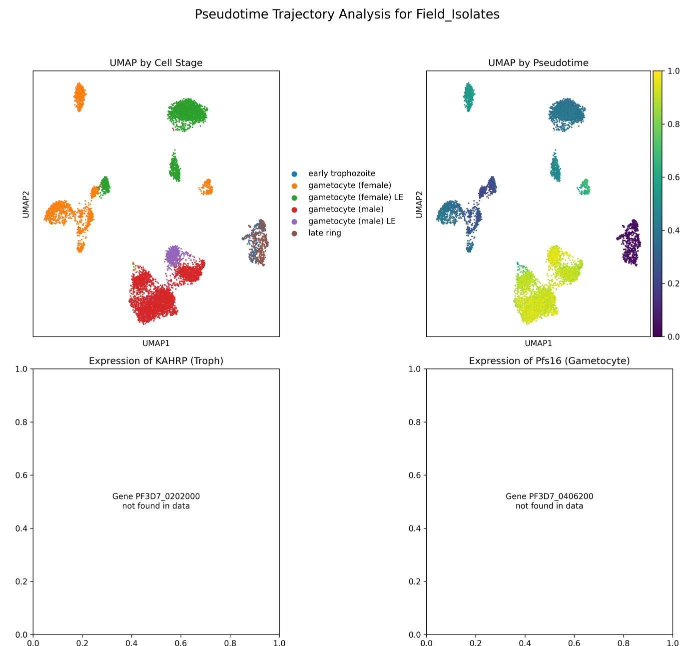

Figure 1. UMAP visualization of *P. falciparum* field isolates, showing clustering of cells by annotated stage and by diffusion pseudotime (DPT). The trajectory originates in late ring stages and progresses through early trophozoites towards gametocytes, capturing the developmental progression from asexual to sexual stages. The bottom panels show that expression data for KAHRP and Pfs16 were not found.

The analysis of stage locations along the pseudotime revealed a critical transition window corresponding to the commitment to gametocytogenesis. The median pseudotime for 'early trophozoite' cells was 0.028, while the median for the combined 'gametocyte' population was 0.794. We therefore defined the "Trophozoite-to-Gametocyte" transition as the pseudotime interval between these points. Our algorithm searched for peaks occurring within this window, representing transcriptional events that happen after the ring stage but precede or coincide with the full maturation of gametocytes.

From our list of candidates, three genes robustly met our stringent criteria, exhibiting significant and transient expression peaks within the Trophozoite-to-Gametocyte transition window. These genes, whose expression profiles are shown in Figure 2, are the

ApiAP2 transcription factor **AP2-G** (`PF3D7-1222600`), a putative **Protein Phosphatase 2C (PP2C)** (`PF3D7-1133400`), and a **FIKK family protein kinase** (`PF3D7-1317100`).

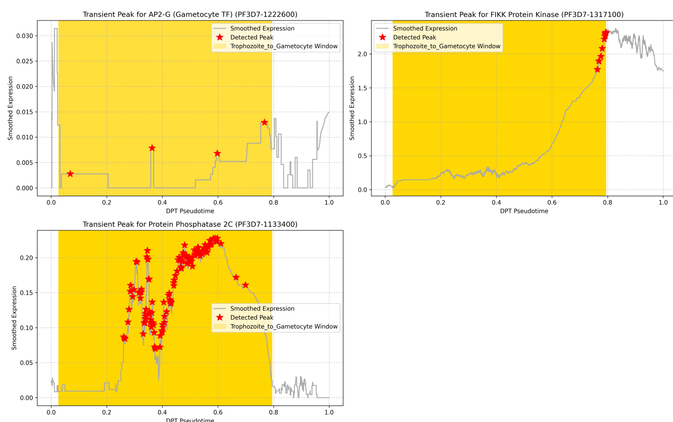

Figure 2. Expression profiles along pseudotime showing transient peaks for AP2-G, Protein Phosphatase 2C, and a FIKK family protein kinase. These peaks, occurring within the Trophozoite-to-Gametocyte transition window, highlight potential regulatory events preceding gametocytogenesis.

3.2.1. *AP2-G: Master Regulator of Gametocytogenesis*

The detection of AP2-G serves as a powerful positive control for our methodology. AP2-G is the widely recognized master switch for sexual commitment in *P. falciparum*. Its expression is tightly repressed during the asexual cycle and is transiently activated in a subset of trophozoites to initiate the entire gametocyte development program. Our analysis captured this event perfectly, identifying a sharp peak for AP2-G very early in the transition window (pseudotime 0.069), as seen in Figure 2. The calculated fold-change was infinite, as the gene's baseline expression across the trajectory was effectively zero, underscoring its role as a tightly controlled, switch-like regulator.

3.2.2. *PP2C and FIKK Kinase: Potential Regulators of Gametocyte Maturation*

The other two regulators, PP2C and the FIKK kinase, showed peaks at later points in pseudotime, suggesting roles in the subsequent progression and maturation of gametocytes rather than the initial commitment. As shown in Figure 2, the PP2C peaked at pseudotime 0.598 with a fold-change of 10.63, while the FIKK protein kinase peaked at pseudotime 0.793 with a fold-change of 4.02.

3.3. *Patient-Specific Heterogeneity in Regulatory Gene Activation*

A key advantage of analyzing field isolates is the ability to explore transcriptional heterogeneity across different natural infections. Having identified three candidate regulators, we investigated whether their activation was uniform across the four patients or if there were patient-specific patterns. For each of the three genes, we identified its most prominent peak (defined by the highest fold-change over baseline) and quantified the proportional contribution of cells from each patient to that peak's local pseudotime window. The results of this analysis are visualized in Figure 3.

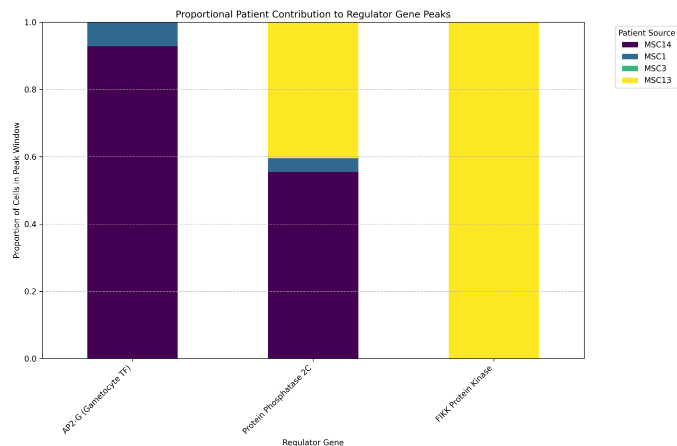

Figure 3. Patient-specific contribution to the transient expression peaks of AP2-G, Protein Phosphatase 2C, and a FIKK kinase. The stacked bar chart shows the proportion of cells from each patient within the pseudotime window of each regulator's peak, revealing that the activation of these regulators is not uniform across patients, suggesting host-specific regulation of parasite development.

3.3.1. *AP2-G Activation is Dominated by a Single Patient*

The transient peak of the master regulator AP2-G, which signals the commitment to sexual development, was overwhelmingly driven by cells from a single patient. As shown in Figure 3, approximately 93% of the cells within the AP2-G peak window originated from patient **MSC14**, with a minor contribution from MSC1 ($\sim$7%) and none from MSC3 or MSC13. This finding suggests that in this cohort, the active process of committing to gametocytogenesis was predominantly occurring in the parasite population of one specific host. This could reflect differences in the host immune environment (e.g., factors that induce sexual commitment as a stress response) or intrinsic properties of the parasite strains within that patient.

3.3.2. *Distinct Patient Coalitions Drive Later Regulatory Events*

The regulatory events occurring later in gametocyte development also showed strong patient-specific signatures, but with different patient combinations. According to Figure 3, the peak for the **Protein Phosphatase 2C** (PF3D7-1133400), which occurs mid-trajectory (pseudotime ∼0.60), was primarily composed of cells from two patients: **MSC14** (∼55%) and **MSC13** (∼41%). This pattern is distinct from the AP2-G activation, suggesting that a different set of host conditions or parasite programs governs this mid-gametocytogenesis regulatory event. The transient activation of this phosphatase may be involved in controlling the progression through gametocyte stages (e.g., I-V), and its prominence in MSC13 and MSC14 suggests this process was particularly active in those infections.

3.3.3. *FIKK Kinase Peak is Exclusive to One Patient*

The most extreme case of patient specificity was observed for the **FIKK kinase** (PF3D7-1317100). Its transient expression peak, occurring late in the trajectory near mature gametocytes (pseudotime ∼0.79), was composed **exclusively (100%) of cells from patient MSC13**, as can be seen in Figure 3. The FIKK kinase family is known to be involved in parasite signaling and remodeling of the host erythrocyte. The exclusive activation of this specific FIKK in parasites from MSC13 points to a highly specific host-parasite interaction or a unique feature of the parasite population within that single patient. This could represent a specific maturation signal, a response to a unique host factor, or preparation for transmission that was only active in that particular infection environment.

3.4. *Summary of Results*

In summary, our analysis of single-cell transcriptomic data from *P. falciparum* field isolates has revealed key insights into the regulatory mechanisms governing gametocytogenesis in vivo. We successfully reconstructed the developmental trajectory of parasites within human hosts (Figure 1) and identified three transiently expressed regulators—AP2-G, a PP2C phosphatase, and a FIKK kinase (Figure 2)—that play critical roles in the commitment to and progression through gametocyte development. Furthermore, we uncovered striking patient-specific heterogeneity in the activation of these regulatory pathways (Figure 3), highlighting the plasticity of parasite development in response to the diverse host environments encountered in natural infections. The detection of AP2-G validated our approach, while the discovery of the PP2C and FIKK kinase suggests a multi-step regulatory cascade. The patient-specific heterogeneity points to the influence of host-specific factors on parasite development and highlights the need for further research to understand these complex interactions.

4. CONCLUSIONS

4.1. *Summary of Findings*

This study addressed the challenge of understanding the regulatory mechanisms driving *Plasmodium falciparum* gametocytogenesis *in vivo*, a process critical for malaria transmission. By analyzing single-cell RNA sequencing data from patient-derived field isolates, we aimed to identify transiently expressed transcriptional regulators orchestrating stage transitions, focusing on patient-specific heterogeneity.

We reconstructed the developmental pseudotime trajectory of parasites from four asymptomatic individuals, using 8,067 high-quality single cells and focusing on 1,510 highly variable genes. We then systematically identified genes exhibiting significant, transient expression peaks preceding major stage transitions, with a focus on transcription factors, kinases, and phosphatases.

Our analysis revealed patient-specific heterogeneity in the activation of key regulators during gametocytogenesis, including the master regulator AP2-G, a protein phosphatase 2C (PP2C), and a FIKK family protein kinase. Specifically, AP2-G activation was dominated by a single patient (MSC14), the PP2C peak was driven by a coalition of MSC14 and MSC13, and the FIKK kinase peak was exclusive to MSC13.

4.2. *Implications and Future Directions*

These findings highlight the plasticity of parasite development in response to varying host environments. The identification of AP2-G validates our approach, while the discovery of the PP2C and FIKK kinase suggests a multi-step regulatory cascade in gametocyte maturation. The patient-specific heterogeneity points to the influence of host-specific factors on parasite development, such as immune responses or drug exposure.

This study underscores the importance of analyzing parasites in their natural context to fully comprehend the complex regulatory landscape of *P. falciparum*. Future research should focus on elucidating the specific host factors that drive the observed patient-specific differences in regulator activation. Furthermore, functional studies are needed to validate the roles of the identified PP2C and FIKK kinase in gametocytogenesis and to explore their potential as targets for interventions aimed at disrupting malaria transmission. This study contributes to a deeper understanding of the regulatory mechanisms governing *P. falciparum* development *in vivo* and provides a foundation for future research aimed at developing novel transmission-blocking strategies.