Genomic Analysis of Colombian Leishmania panamensis strains with different level of virulence

Daniel Alfonso Urrea, Jorge Duitama, Hideo Imamura, Juan F. Álzate, Juanita Gil, Natalia Muñoz, Janny Alexander Villa, Jean-Claude Dujardin, José R. Ramirez-Pineda, Omar Triana-







Supplementary Figure 1. Row data of lesion measure in the ear per week for each strain in both experiment done. Each line represents the follow performed on each BALB/c mice.

Lesion area measure

Kruskal-Wallis test for equal medians

| H (chi2): | 69,31 | |
|------------------------|--------------|------------------------|
| Hc (tie corrected): | 69,99 | |
| p (same): | 4,28E-15 | |
| There is a significant | difference l | between sample medians |

Mann-Whitney pairwise comparisons with Bonferroni correction of p values

| | UA946 | UA140 | UA1114 | UA1511 |
|--------|----------|----------|----------|----------|
| UA946 | | 9,88E-14 | 0,004352 | 0,002041 |
| UA140 | 9,88E-14 | | 2,87E-06 | 3,59E-08 |
| UA1114 | 0,004352 | 2,87E-06 | | 1 |
| UA1511 | 0,002041 | 3,59E-08 | 1 | |

Lesion intensity scale

Kruskal-Wallis test for equal medians

| H (chi2): | 42,41 | | |
|-------------------------|----------|--|--|
| Hc (tie corrected): | 46,32 | | |
| p (same): | 4,85E-10 | | |
| The sup to a stantfloor | | hat we have a second se | |

There is a significant difference between sample medians

Mann-Whitney pairwise comparisons with Bonferroni correction of p values

| | UA946 | UA140 | UA1114 | UA1511 |
|--------|----------|-----------|-----------|-----------|
| UA946 | | 1,52E-08 | 0,002517 | 0,006404 |
| UA140 | 1,52E-08 | | 0,0001386 | 0,0001283 |
| UA1114 | 0,002517 | 0,0001386 | | 1 |
| UA1511 | 0,006404 | 0,0001283 | 1 | |

Parasitic load

Kruskal-Wallis test for equal medians

| H (chi2): | 8,314 | |
|---------------------------|--------------|-----------------------|
| Hc (tie corrected): | 8,862 | |
| p (same): | 0,03118 | |
| There is a significant of | difference b | etween sample medians |

mere is a significant anterence between sample medians

Mann-Whitney pairwise comparisons with Bonferroni correction of p values

| | UA946 | UA140 | UA1114 | UA1511 |
|--------|---------|---------|---------|---------|
| UA946 | | 0,02173 | 0,09283 | 0,08055 |
| UA140 | 0,02173 | | 0,07839 | 0,1051 |
| UA1114 | 0,09283 | 0,07839 | | 0,6916 |
| UA1511 | 0,08055 | 0,1051 | 0,6916 | |

Supplementary Table1. Kruskal-Wallis statistical test and Mann-Whitney pairwise comparisons between experimental variables for the two experiments performed.



Supplementary Figure 2. Example of visualization of short read alignments for a 1kbp region in which an insertion was predicted within UA946 (lower panel) relative to M2904 (upper panel), using whole genome comparisons. Aligned reads from the *L. panamensis* strain PSC-1 is also shown in the middle panel. Red alignments have predicted insert lengths of more than 1kbp. Reads painted with other colors have their mates aligned to other chromosomes.



Supplementary Figure 3. Illumina reads mapped to the genome of strain UA946 of *L. panamensis*.



Supplementary Figure 4. Venn diagrams for the scenario in which there is more intersection between machine learning methods and for which there is less intersection.



Supplementary Figure 5. Quality test of assembled genome based on the mapping error percentage of reads by position; A) 454 reads. B) Paired-end reads illumina. Media coverage sequencing; C) 454 reads. D) Paired-end reads illumina (program NGSEP).



Supplementary Figure 6. Repetitive regions identified by self blast and self mapping using NGSEP program.



Supplementary Figure 7. Example of visualization of short read alignments for a 1kbp region in which an insertion was predicted within UA946 (lower panel) relative to M2904 (upper panel) using whole genome comparisons. Aligned reads from the *L. panamensis* strain PSC-1 is also shown in the middle panel. Red alignments have predicted insert lengths of more than 1kbp. Reads painted with other colors have their mates aligned to other chromosomes.

| Chr | Chromoso | mes befor | e IMAGE | Chromos | Chromosomes after IMAGE Difference | | Difference | | |
|-------|------------|-----------------------|---------|------------|------------------------------------|-----|------------|--------|-----|
| CIII | Length | Ns | #gaps | Length | ngth Ns #gaps Length Ns | | Ns | #gaps | |
| 1 | 267.023 | 10.844 | 22 | 267.401 | 10.775 | 21 | 378 | 69 | 1 |
| 2 | 296.744 | 35.551 | 18 | 297.007 | 33.487 | 18 | 263 | 2064 | 0 |
| 3 | 387.899 | 10.262 | 14 | 389.201 | 10.216 | 16 | 1.302 | 46 | 2 |
| 4 | 436.471 | 9.163 | 47 | 436.986 | 9.040 | 20 | 515 | 123 | 27 |
| 5 | 476.818 | 30.324 | 27 | 569.282 | 29.424 | 22 | 92.464 | 900 | 5 |
| 6 | 516.202 | 10.246 | 34 | 516.730 | 10.239 | 18 | 528 | 7 | 16 |
| 7 | 565.104 | 21.593 | 19 | 565.747 | 19.040 | 17 | 643 | 2553 | 2 |
| 8 | 446.896 | 21.292 | 18 | 447.970 | 20.699 | 15 | 1074 | 593 | 3 |
| 9 | 582.716 | 32.282 | 24 | 584.259 | 31.633 | 24 | 1543 | 649 | 0 |
| 10 | 526.398 | 45.179 | 16 | 527.106 | 43.313 | 13 | 708 | 1866 | 3 |
| 11 | 592.229 | 25.477 | 24 | 589.864 | 18.352 | 20 | 2365 | 7125 | 4 |
| 12 | 493.387 | 5.804 | 14 | 493.739 | 5.610 | 14 | 352 | 194 | 0 |
| 13 | 615.759 | 6.756 | 16 | 616.788 | 6.895 | 13 | 1029 | 139 | 3 |
| 14 | 651.698 | 86.728 | 42 | 651.698 | 85.161 | 17 | 0 | 1567 | 25 |
| 15 | 614.203 | 19.840 | 18 | 614.686 | 19.422 | 18 | 483 | 418 | 0 |
| 16 | 692.608 | 43.832 | 41 | 693.464 | 43.315 | 26 | 856 | 517 | 15 |
| 17 | 682.174 | 72.101 | 18 | 682.291 | 71.514 | 16 | 117 | 587 | 2 |
| 18 | 687.899 | 17.355 | 20 | 690.206 | 17.239 | 18 | 2307 | 116 | 2 |
| 19 | 665.187 | 32.370 | 19 | 677.420 | 32.121 | 17 | 12233 | 249 | 2 |
| 20 | 2.520.950 | 230.606 | 83 | 2.521.761 | 222.316 | 58 | 811 | 8290 | 25 |
| 21 | 748.705 | 21.347 | 25 | 748.814 | 21.123 | 16 | 109 | 224 | 9 |
| 22 | 668.618 | 34.667 | 55 | 762.856 | 32.295 | 25 | 94238 | 2372 | 30 |
| 23 | 775.951 | 47.379 | 44 | 776.507 | 42.467 | 33 | 556 | 4912 | 11 |
| 24 | 823.052 | 17.427 | 24 | 823.589 | 16.279 | 22 | 537 | 1148 | 2 |
| 25 | 886.525 | 11.916 | 30 | 888.185 | 11.400 | 17 | 1660 | 516 | 13 |
| 26 | 1.026.257 | 13.038 | 23 | 1.026.457 | 10.427 | 15 | 200 | 2611 | 8 |
| 27 | 1.032.823 | 38.679 | 26 | 1.033.693 | 38.123 | 25 | 870 | 556 | 1 |
| 28 | 1.168.439 | 34.150 | 27 | 1.170.028 | 34.428 | 23 | 1589 | 278 | 5 |
| 29 | 1.212.475 | 52.225 | 40 | 1.214.198 | 49.541 | 34 | 1723 | 2684 | 6 |
| 30 | 1.304.861 | 8.162 | 21 | 1.397.265 | 8.061 | 18 | 92404 | 101 | 3 |
| 31 | 1.345.191 | 30.495 | 19 | 1.345.688 | 28.649 | 17 | 497 | 1846 | 2 |
| 32 | 1.534.377 | 26.065 | 40 | 1.534.710 | 24.234 | 21 | 333 | 1831 | 19 |
| 33 | 1.440.683 | 55.622 | 46 | 1.441.563 | 54.233 | 32 | 880 | 1389 | 14 |
| 34 | 1.986.720 | 71.843 | 60 | 1.985.663 | 65.598 | 49 | 1057 | 6245 | 11 |
| 35 | 2.619.390 | 67.5 <mark>6</mark> 8 | 116 | 2.621.291 | 55.289 | 93 | 1901 | 12279 | 23 |
| | | | | | | | | | |
| Total | 31.292.432 | 1.298.188 | 1130 | 31.604.113 | 1.231.958 | 841 | 318.525 | 67.064 | 294 |

Supplementary Table2. Use of the IMAGE tool for the closure of gaps in the genome of strain UA946 of *L. panamensis*.

| Geographical origin | | Old World | | New World | | | | |
|------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|---------------------|---------------------|
| Clinical form | VL CL | | | CL / | DCL | | | CL / MCL |
| Subgenus | Leishmania | | | | | | | Viannia |
| Species | L. infantum | L. donovani | L. major | L. mexicana | L. amazonensis | L. braziliensis | L. guyanensis | L. naiffi |
| Strain | JPCM5 | BPK282/0cl4-LV9 | Friedlin | U1103 | M2269 | M2904 | LgCL085 | LnCL223 |
| Sequencing | Sanger/Illumina | 454/Illumina | Sanger/Illumina | Sanger/Illumina | 454/Illumina | Sanger/Illumina | Illumina | Illumina |
| Size | 32,101,728 | 32,444,998 | 32,855,089 | 32,108,741 | 32,444,998 | 31,997,773 | 31,014,322 | 30,341,095 |
| Contigs/scaffolds | 562 | 2.154 | 36 | 375 | 2.627 | 1.041 | 2.800 | 6.530 |
| Chromosomes | 36 | 36 | 36 | 34 | 34 | 35 | 35 | 35 |
| Depth coverage | 80X | 74X | 56X | 112X | 74X | 107X | 56X | 36X |
| Heterozygous SNPs | 629 | 3549 | 297 | 12531 | N/A | 44588 | 705 | 14739 |
| Overall GC content (%) | 59,6 | 57,3 | 59,7 | 59,7 | 58,5 | 57,8 | 57,34 | 57,53 |
| Coding GC content (%) | 62.4 | 61 | 62.5 | 61.23 | 61 | 60.3 | N/A | N/A |
| Genes | 8241 | 8252 | 8412 | 8250 | 8100 | 8359 | 8376 | 8262 |
| Pseudogenes | 41 | N/A | 97 | 98 | 36 | 195 | 51 | 59 |
| Poforonco | Peacock et al 2007 | Downing et al 2011 | Peacock et al 2007 | Tschoeke et al2014 | Tschoeke et al2014 | Peacock et al 2007 | Coughlan et al 2018 | Coughlan et al 2018 |
| Kelelelice | Rogers et al 2011 | Real et al 2015 | Rogers et al 2011 | Cougnian of al 2010 | Couginan et al 2010 |

VL (visceral leishmaniases), CL (cutaneous leishmaniases), DCL (diffuse cutaneous leishmaniasis), MCL (mucocutaneous leishmaniasis)

N/A, not available

Supplementary Table3. Genomic characteristics of the strains of *Leishmania* spp., pathogenic for humans reported. The gray shadow shows the contribution generated in this work with the virulent strain UA946 of *L. panamensis*.