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Coronavirus Pandemic

The first reported case of candidemia caused by the novel *Candida tropicalis* diploid sequence type 1515

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Abstract

Introduction: Since the dawn of the new millennium, *Candida* species have been increasingly implicated as a cause of both healthcare-associated as well as opportunistic yeast infections, due to the widespread use of indwelling medical devices, total parenteral nutrition, systemic corticosteroids, cytotoxic chemotherapy, and broad-spectrum antibiotics. *Candida tropicalis* is a pathogenic *Candida* species associated with considerable morbidity, mortality, and drug resistance issues on a global scale.

Methodology: We report a case of a 43-year-old man who was admitted to our hospital for further management of severe coronavirus disease 2019 (COVID-19) pneumonia. During his stay in the ward, he received systemic corticosteroids for a total duration of 32 days. A broad-spectrum antibiotic (piperacillin-tazobactam) was also given due to copious amounts of tracheostomy secretions.

Results: The patient's fever recurred following an afebrile interval of 11 days, and *C. tropicalis* was cultured from his blood. The yeast was highly resistant to fluconazole and voriconazole but remained susceptible to echinocandins. Unfortunately, the patient was unable to receive any echinocandin and eventually succumbed to candidemia.

Conclusions: Multilocus sequence typing was used to characterize *C. tropicalis* as a novel diploid sequence type (i.e., 1515) that has not been previously reported.

Key words: *Candida tropicalis*; candidemia; COVID-19; multilocus sequence typing.

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Introduction

Over 200 species of *Candida* have been described to date; however, 90% of invasive human mycoses are attributable to just 5 species, viz. *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, and *Pichia kudriavzevii* (formerly *Candida krusei*) [1]. The designation of a yeast as a non-albicans *Candida* (NAC) species is not done merely for academic interests, but also because of the stigma of antifungal resistance (namely to azole compounds) typically associated with it. Hence, it is conceivable that as a NAC species, *C. tropicalis* is also notorious for its decreased susceptibility to fluconazole [2]. A recent Malaysian study found that approximately 30% of *C. tropicalis* isolates were resistant to fluconazole, while susceptibility rates to amphotericin B (AmB) and echinocandins were still at 100% [3]. On top of this, the mortality rate of NAC fungemia generally exceeds that caused by *C. albicans* (e.g., the mortality rate is 83% for *C. glabrata* and 81% for *C. tropicalis* vs. 63% for *C. albicans*) [4]. We report a case of fatal *C. tropicalis* fungemia in a coronavirus disease 2019 (COVID-19) patient who received prolonged systemic corticosteroids and broad-spectrum antibiotics during his stay in hospital. This is the first reported case of candidemia caused by the novel *C. tropicalis* diploid sequence type (DST) 1515.

Case report

A 43-year-old man with a 1-year history of traumatic brain injury was admitted to our hospital for further management of severe COVID-19 pneumonia requiring supplemental oxygen. He had been bedbound due to the neurological deficits resulting from the traumatic brain injury and had to live with a tracheostomy and a percutaneous endoscopic gastrostomy tube ever since. A stage II pressure sore had also developed at his sacral region during this 1-year period. Additionally, oral amoxicillin-clavulanate had been prescribed on at least four different occasions during his follow-ups in the outpatient clinics.

Table 1. Antifungal minimal inhibitory concentration (MIC) results.

Antifungal agent	MIC (µg/mL)
Amphotericin B	1
Fluconazole	128
Itraconazole	0.5
Voriconazole	4
Posaconazole	0.5
Anidulafungin	0.12
Caspofungin	0.25
Micafungin	0.03
Flucytosine	0.12

When examined at the emergency department, the patient had blood pressure of 103/77 mmHg, heart rate of 106 beats per minute, respiratory rate of 28 breaths per minute, temperature of 38.6 °C, and room air oxygen saturation of 89%. He was initially nursed in our hospital's COVID-19 intensive care unit (COVID-19 ICU), during which intravenous methylprednisolone (40 mg twice daily) was administered. Intravenous piperacillin-tazobactam (4.5 g thrice daily) was also administered because his tracheostomy produced copious amounts of secretions. A bacterial pathogen could not be identified at this juncture because his tracheal aspirate culture had mixed growth, and his blood cultures were negative. Nevertheless, the methylprednisolone dose was doubled to 80 mg twice daily and piperacillin-tazobactam was administered for a total of 8 days. No central venous catheter was inserted during his stay in the COVID-19 ICU and after spending 18 days there, he was stable enough to be transferred out to the general medical ward. The intravenous methylprednisolone was replaced with oral prednisolone at a dose of 35 mg daily to continue the steroid therapy.

Unfortunately, after being afebrile for just 11 days, a recurrence of fever was documented. The C-reactive protein level had by now peaked to 3.72 mg/dL (following a nadir of 0.93 mg/dL just prior to his transfer out to the general ward). A repeat blood culture was prescribed and the administration of intravenous piperacillin-tazobactam was restarted. Unfortunately, the blood was now positive for a fungal pathogen. The fungus was identified biochemically as the ascomycetous yeast *Candida tropicalis* by the ID 32 C kit (bioMérieux, Lyon, France) with an identification percentage of 95% (numerical profile: 5167340115; good identification). Piperacillin-tazobactam was discontinued, the steroid therapy was withheld (after being administered for a total duration of 32 days), and intravenous fluconazole (administered as a statim dose of 800 mg followed by a daily dose of 400 mg) was commenced while awaiting the results of antifungal susceptibility testing. We obtained minimal inhibitory concentration (MIC) readings for the nine antifungal agents using the colorimetric broth microdilution kit Sensititre YeastOne YO10 (TREK Diagnostic Systems, Oakwood Village, USA; Table 1). The patient had poor urine output and tachycardia (heart rate of up to 140 beats per minute). Therefore, AmB, which is a nephrotoxic and potentially cardiotoxic drug, was not administered. An echinocandin was also not administered due to financial constraints, despite susceptible MIC readings for all the three agents tested. The patient passed away 10 days after the yeast was first detected in his blood.

Table 2. Multilocus sequence typing (MLST) result for *Candida tropicalis.*

Housekeeping gene	Alleles	Diploid sequence type
ICL1	1	1515 (novel)
MDR1	90	
SAPT2	63	
SAPT4	10	
XYR1	24	
ZWF1a	3	

Following the patient's demise, multilocus sequence typing (MLST) of the *C. tropicalis* isolate was attempted through the whole-genome sequencing method. The DNA was extracted using the Zymo Quick-DNA miniprep kit (Zymo Research, Irvine, USA). Genomic DNA was fragmented and processed using the NEBNext Ultra II DNA library preparation kit (New England Biolabs, Ipswich, USA) and paired-end sequenced on a NovaSEQ6000 (Illumina, San Diego, USA) platform (2×150bp) according to the manufacturer's recommendations for whole-genome analysis. Sequencing reads were trimmed for adapter and quality using the Fastp tool and the resulting reads were assembled de novo with the Masurca v4.1.0 tool [5]. The genome completeness and assembly statistics were computed using the BUSCO 5.4.3 and QUAST5 tools, respectively [6,7]. The trimmed reads were subjected to alignment against the assembled genome using Bowtie2 v2.4.4 to construct a degenerate rendition of the genome assembly that highlights regions exhibiting heterozygosity [8]. Subsequently, consensus calling was performed, and the degenerate base was incorporated into regions exhibiting heterozygosity using BCFtools v1.13 [9]. The identification of genomic regions encoding the standard six housekeeping genes (i.e., *ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1*, and *ZWF1a*) used for MLST profiling was accomplished with BlastN search and extracted with BEDtools. Supplementary File 1 lists the

housekeeping gene sequences of the yeast isolate. The retrieved loci were then subjected to analysis using PubMLST (http://pubmlst.org/ctropicalis/) for allele identification and assignment of DST [10]. The isolate's MLST result is presented in Table 2. Its raw whole genome sequence data has been deposited in the NCBI database under BioProject number PRJNA1000249 and BioSample number SAMN36792961.

Discussion

Prolonged steroid use in COVID-19 management has been a subject of tremendous interest due to its immunosuppressive effects. Several studies have explored the association between extended steroid treatment and the development of candidemia. A recent meta-analysis suggested a potential link between prolonged corticosteroid therapy and an increased incidence of candidemia in COVID-19 patients [11]. The immunomodulatory effects of steroids may impair the host's ability to control or combat fungal infections, leading to systemic candidiasis. At the same time, it has also been proposed that while steroids are associated with an elevated risk of secondary infections, including candidemia, the benefits of reducing inflammation in severe COVID-19 cases may outweigh the potential drawbacks [12]. This highlights the complexity of the fine balance between the therapeutic benefits of steroids and the risk of infectious complications. Certainly, long-term use and higher doses of steroids can amplify the risk, as was the case with our patient who was on steroid therapy for more than a month. Further research is needed to elucidate the specific mechanisms underlying this association and to develop strategies for mitigating the risk of fungal infections in individuals undergoing extended steroid therapy for severe COVID-19 infection.

Apart from steroid therapy, another notable risk factor for candidemia in our patient was the administration of broad-spectrum antibiotics (i.e., amoxicillin-clavulanate and piperacillin-tazobactam). It has been long established that exposure to antibiotics (particularly those which are excreted in the feces) can alter the commensal bacteria in the host's gut, resulting in increased colonization (and the subsequent infection) with more antibiotic-resistant pathogens typically found in healthcare institutions [13]. It is imperative to note that beta-lactam/beta-lactamase inhibitor combination agents have significant anaerobic coverage, which further amplifies the alteration of the gut flora [14]. Hebert *et al.* have specifically found that piperacillin-tazobactam increased the risk of bloodstream infections with *Candida* spp. that are fluconazole non-susceptible (defined by the authors as a fluconazole MIC of at least 16 μg/mL). The Clinical and Laboratory Standards Institute (CLSI) has since revised the designation of fluconazole non-susceptibility (which includes the "susceptible-dose dependent" and "resistant" categories) from ≥16 μg/mL to just ≥4 μg/mL for most *Candida* spp. in the 2022 edition of its M27M44S document [15]. Not only was our isolate resistant to fluconazole, its MIC of 128 μg/mL was only one dilution below the highest measurable limit of the Sensititre kit.

MLST is a method that has been extensively used in the molecular epidemiology of *C. tropicalis* [16]. MLST has very high discriminatory power (≥ 99%) and excellent reproducibility and is based on the analysis of single nucleotide polymorphisms of housekeeping gene fragments that are not under selective pressures [17]. The *C. tropicalis* MLST system proposed by Tavanti *et al*. has been in use for nearly two decades and entails the comparisons of the six housekeeping genes that are listed in Table 2 [17]. The combination of results from analyzing these gene fragments generates DSTs that can be utilized to quantify similarities and putative genetic relationships between various *C. tropicalis* isolates [18]. The *C. tropicalis* MLST database facilitates comparing strains and populations from various laboratories from around the globe, revealing their diverse geographical origins, anatomical sources, main underlying diseases, and antifungal susceptibility profiles. There are specific DSTs which are notorious for their antifungal resistance, such as DST 140 which was reported in Taiwan and possessed fluconazole MICs of at least 64 μg/mL [19]. DST 164, which was also first reported in Taiwan, had flucytosine MICs of at least 8 μg/mL [18]. Thus, it appears that *C. tropicalis* isolates of the same DST represent a common clone that has undergone significant mutations to endure drug selection pressures or adapt to different geographical environments [18]. DST 1515, which is being reported for the very first time, was isolated in Kuala Lumpur. It is highly resistant to both fluconazole and voriconazole, but remains susceptible to echinocandins, based on current CLSI breakpoints [15]. Despite low MIC values, resistance could not be called for posaconazole, itraconazole, flucytosine, and AmB due to the absence of agent-specific CLSI breakpoints [15].

The Infectious Diseases Society of America (IDSA) strongly recommends echinocandins for the treatment of candidemia in both neutropenic and non-neutropenic patients [20]. Unfortunately, due to cost issues (generic echinocandin brands were not yet available in our

hospital's formulary when the patient's candidemia was diagnosed; and a single vial of an echinocandin costs nearly 50 times that of fluconazole), it is doubtful that the society's recommendation on echinocandin drugs usage can be fully adopted by institutions with limited resources. In addition, since echinocandins are also the first-line therapy for specific NAC species such as *Candida auris*, its already limited stockpile available to treat infections caused by *C. tropicalis* would shrink further as the prevalence of azole-resistant NAC species increases [3]. AmB, to which our isolate had a reasonably low MIC of 1 μg/mL, is available as either a conventional (i.e., deoxycholate) or a lipid formulation. In our hospital, 1 vial of conventional AmB costs approximately 15 times that of fluconazole, while 1 vial of the lipid formulation is 55 times more expensive than fluconazole. A key advantage of a lipid AmB formulation is its significantly lower nephrotoxicity compared to conventional AmB [21]. Unfortunately, we were unable to administer the conventional and lipid formulations of AmB due to the patient's existing nephrotoxicity and financial constraints, respectively. Despite a low MIC of just 0.12 μg/mL, flucytosine was also not given because this drug is generally administered in combination with another antifungal agent to curtail the rapid emergence of drug resistance with monotherapy [20].

Conclusions

Any case of *C. tropicalis* fungemia should be managed with prudence. Antifungal susceptibility testing is mandatory for such cases due to the unpredictability of antifungal susceptibility, particularly to lower costing drugs such as fluconazole. Key risk factors such as the need for prolonged systemic corticosteroid use and/or broad-spectrum antibiotic administration should be addressed periodically, with the aim of discontinuing these agents at the earliest opportunity. While MLST is not necessary for all cases of *C. tropicalis* fungemia, it can be a valuable outbreak investigation tool in the event of a deluge of azole-resistant isolates in a healthcare institution.

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References

1. Yesudhason BL, Mohanram K (2015) Candida tropicalis as a predominant isolate from clinical specimens and its antifungal susceptibility pattern in a tertiary care hospital in Southern India. J Clin Diagn Res 9: DC14–DC16. doi: 10.7860/JCDR/2015/13460.6208.
2. Deorukhkar SC, Saini S, Mathew S (2014) Virulence factors contributing to pathogenicity of Candida tropicalis and its antifungal susceptibility profile. Int J Microbiol: 456878. doi: 10.1155/2014/456878.
3. Navarathinam SD, Neoh HM, Tan TL, Wahab AA, Tzar MN, Ding CH (2023) Antifungal susceptibility profile and biofilm-producing capability of Candida tropicalis isolates in a tertiary medical centre. Malays J Pathol 45: 417–424.
4. Megri Y, Arastehfar A, Boekhout T, Daneshnia F, Hörtnagl C, Sartori B, Hafez A, Pan W, Lass-Flörl C, Hamrioui B (2020) Candida tropicalis is the most prevalent yeast species causing candidemia in Algeria: the urgent need for antifungal stewardship and infection control measures. Antimicrob Resist Infect Control 9: 50. doi: 10.1186/s13756-020-00710-z.
5. Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA (2013) The MaSuRCA genome assembler. Bioinformatics 29: 2669–2677. doi: 10.1093/bioinformatics/btt476.
6. Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUAST: quality assessment tool for genome assemblies. Bioinformatics 29: 1072–1075. doi: 10.1093/bioinformatics/btt086.
7. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM (2015) BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31: 3210–3212. doi: 10.1093/bioinformatics/btv351.
8. Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9: 357–359. doi: 10.1038/nmeth.1923.
9. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H (2021) Twelve years of SAMtools and BCFtools. Gigascience 10: giab008. doi: 10.1093/gigascience/giab008.
10. Jolley KA, Bray JE, Maiden MCJ (2018) Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 3: 124. doi: 10.12688/wellcomeopenres.14826.1.
11. Li Z, Denning DW (2023) The impact of corticosteroids on the outcome of fungal disease: a systematic review and meta-analysis. Curr Fungal Infect Rep 17: 54–70. doi: 10.1007/s12281-023-00456-2.
12. Langarizadeh MA, Ranjbar Tavakoli M, Abiri A, Ghasempour A, Rezaei M, Ameri A (2021) A review on function and side effects of systemic corticosteroids used in high-grade COVID-19 to prevent cytokine storms. EXCLI J 20: 339–365. doi: 10.17179/excli2020-3196.
13. Hebert C, Villaran R, Tolentino J, Best L, Boonlayangoor S, Pitrak D, Lin M, Weber SG (2010) Prior antimicrobial exposure and the risk for bloodstream infection with fluconazole-non-susceptible Candida strains. Scand J Infect Dis 42: 506–509. doi: 10.3109/00365541003699631.
14. Pultz NJ, Stiefel U, Ghannoum M, Helfand MS, Donskey CJ (2005) Effect of parenteral antibiotic administration on establishment of intestinal colonization by Candida glabrata in adult mice. Antimicrob Agents Chemother 49: 438–440. doi: 10.1128/AAC.49.1.438-440.2005.
15. CLSI (2022) Performance standards for antifungal susceptibility testing of yeasts. 3rd ed. CLSI supplement

M27M44S. Wayne, PA: Clinical and Laboratory Standards Institute.
16. Wang Q, Tang D, Tang K, Guo J, Huang Y, Li C (2020) Multilocus sequence typing reveals clonality of fluconazole-nonsusceptible Candida tropicalis: a study from Wuhan to the global. Front Microbiol 11: 554249. doi: 10.3389/fmicb.2020.554249.
17. Tavanti A, Davidson AD, Johnson EM, Maiden MC, Shaw DJ, Gow NA, Odds FC (2005) Multilocus sequence typing for differentiation of strains of Candida tropicalis. J Clin Microbiol 43: 5593–5600. doi: 10.1128/JCM.43.11.5593-5600.2005.
18. Chen KW, Chen YC, Lin YH, Chou HH, Li SY (2009) The molecular epidemiology of serial Candida tropicalis isolates from ICU patients as revealed by multilocus sequence typing and pulsed-field gel electrophoresis. Infect Genet Evol 9: 912–920. doi: 10.1016/j.meegid.2009.06.011.
19. Li SY, Yang YL, Lin YH, Ko HC, Wang AH, Chen KW, Wang CW, Chi H, Lo HJ (2009) Two closely related fluconazole-resistant Candida tropicalis clones circulating in Taiwan from 1999 to 2006. Microb Drug Resist 15: 205–210. doi: 10.1089/mdr.2009.0915.
20. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD (2016) Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 62: e1–e50. doi: 10.1093/cid/civ933.
21. Hamill RJ (2013) Amphotericin B formulations: a comparative review of efficacy and toxicity. Drugs 73: 919–934. doi: 10.1007/s40265-013-0069-4.

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Conflict of interests: No conflict of interests is declared.

Annex - Supplementary Materials

Supplementary File 1. *Candida tropicalis* DST 1515 housekeeping gene sequences.

ICL1
CGAAGCTGTCATTGATGAAATCAAGGCTGGTAACTACTCCAATAAAGAAGCTTTGATTAAACAATTCACCGACA
AAGTTAACCCATTGTCCTGTACTTCTCACAAAGAAGCTAAGAAATTGGCCAAAGAATTGACTGGTAAAGATATTT
ACTTCAACTGGGATGTTGCTAGAGCCAGAGAAGGTTACTACAGATACCAAGGTGGTACTCAATGTGCCGTTATG
AGAGGTAGAGCTTTTGCTCCATACGCTGATTTAATCTGGATGGAATCTGCTTTGCCAGACTACAACCAAGCTAAA
GAATTTGCTGACGGTGTTAAAGCTGCTGTTCCAGACCAATGGTTGGCTTACAACTTGTCTCCATCTTTCAACTGG
AACAAAGCTATGCCAGCCGATGAACAAGAAACTTATATCAAGAGATTGGGTAAATTGGGTTACGTATGGCAATT

MDR1
TGATGGTGAAGTTGAAAATTCCAAAATGACAACTCATGAATTGATTGTTGATACTTTATGGAGACCATTGGAGAT
TACAATCATGGAACCGGTTGTTTTGTTGATTGATATTTACATTGCTATGGTTTACAGTATTCTTTATCTTTTCTTTG
AAGTTTTCCCAATTTATTTTGTTGGAGTTAGAGGATTTACTTTGGTTGAACTTGGTACCACTTTCTTTTCCGTGTTG
ATTGGTATTGTTGTTGCCTGYTCTATTTACTTRCCTATCATCAAACGAATTTTCACTGATAGAATTCTCAGAAAAG
AACARGTTTTCCCAGAAGTTTTCATTCCATTAGCTATAGTTGGAGGTTGTTTGTTAACCGGWGGACTATTCATAT
TTGGATGGTCAGCAACTAGAACTACTCATTGGATTGGGCCYTTAT

SAPT2
CTGGTGTCCTTAGTTTAGAGCTTTATGTTAACAGGAATCATGATGATAGTAATTTCACTATTGGTCCCCACTTTGT
TGTTAATGAATATTCTAAGAGAGATGATTATATTAGTGTTGAATTATACAATGAGCAAGTTACTTACTCTGCAAA
TATCACCGTTGGATCAAATAGTCAGAAGCAGAATGTTATTGTTGATACYGGATCCTCTGATTTATGGGTTGTTGA
TTCCAGTGCAAATTGTCAAGAGAAATCCGGATACTCCTCTGATTATTGCTTCAGTGGGGGCACATACGATCCATC
AAGTTCTTCCACAATCCAAGAGTTGGGTAAATCTTTCAACATTAGATATGGAGATGGTTCTTCTTCTAGTGGTAC
CTGGGTCAAAGACACAGTTGGAATCAATGGTGCAATTATTTTAAACCAACAGTTTGGTGATGTGAACTCAACTTC
TGTGTCACAAGGTATATTGGGTATTGGCTTGGATACCAATGAGTCAACTGATACCATCTATGAAAATTTTCCAA

SAPT4
CATTATTAAAACGCAAAAGAATATCGTTCCCAATGAGAATATCATTGTTTCTAAAAGACAACCAGTTCCTGTAAC
TTTGATAAAGGAACAAATAGCTTATGCGGCCGAGATAACTATTGGTTCAAATAAYCARAAACAAACAGTTATTA
TTGATACTGGATCCTCTGATTTATGGGTAGTTGACAAAAATGCCACGTGTGTTCGTAGATTTGAACAACAAGTGC
AAGATTTTTGTAAAGCAAATGGAACGTATGATCCAATTACATCTAGTTCTGCTAAAAAATTAGGAACAGTTTTTG
ATATTAGCTATGGAGATAAAACCAATTCTCTGGGAAATTGGTATAAGGATACTATTAAGATTGGTGGGATTACTA
TTACTRACCAACAATT

XYR1
TCTACAATGCYATYAAAACTGGTTACAGATTATTTGATGGTGCTGAAGATTACGGTAAYGAAAAAGAAGTTGGT
GAAGGTATYAACAGAGCCATTAAAGAWGGATTAGTTAAAAGAGAAGAATTATTCATCACTTCTAAATTATGGA
ACAATTTCCATGATCCAAAGAATGTTGAAACTGCTTTAAACAAAACTTTAAGTGACTTGAACTTGGAYTATGTTG
ATTTATTCTTGATTCATTTYCCAATTGCTTTTAAATTTGTTCCAATTGAAGAAAAATACCCACCTGGTTTCTACTG
TGGTGATGGTGATAACTTCCACTATGAAGATGTTCCATTATTAGAYACTTGGAAAGCTTTGGAAAAATTGGT

ZWF1a
TGCCTTGTTTGGTTTGTTCAGAGAAAAACAATTGCCTTCAACTGTTCAAATCATTGGTTATGCTAGATCTCATTTG
TCTGATAAGGACTTTAAAGACAGAATTTCCTCCCATTTCAAAGGTGGTGATGACAAAACTAAAGAAGATTTCTTG
AACTTGTGTTCTTATATCAGTGATCCATATGATACCGATGAAGGTTACAAGAAATTGGAAGCTCGTTGTCAAGAA
TATGAAAGCAAACACAATGTTAAAGTTCCAGAAAGATTATTCTACTTGGCCTTGCCTCCATCTGTTTTCCACACT
GTTTGTGAACAAGTTAAAAAGAATGTCTATCCAAAAGATGGTAAACTCAGAATCATTATTGAAAAACCATTTGG
CCGTGATTTGGAAACTTACCGTGAATTGCAAAAACAAATCTCCCCATTGTTCACTGAAGATGAAGTTTACAGAAT
TGACCACTACTTGGGTAAAGAAATGGTTAAGAACTTGTTGGTTTTAAGATTTGGTAAYGAATTGTTCAGT