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Research Article

Efficacy of Aromatase Inhibitors for Azoospermia Caused by AZFc Microdeletion: A Cross-Sectional Descriptive Research Study in Chinese Population

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Background. Aromatase inhibitors (AIs) can significantly improve semen parameters in infertile men. In this study, we investigated the efficacy of AIs for azoospermia in a Chinese population with AZFc microdeletion. *Aims*. Patients with AZFc microdeletion who were treated with AIs were analyzed retrospectively by collecting clinical data, including their hormone profile and treatment outcome. Patients were divided into those with sperm in their semen after AI treatment (group A) and those without sperm in their semen after AI treatment (group B). *Results*. The rate of Y chromosome AZF microdeletions was 9.30% (313/3364) from March 2015 to March 2021, among which patients with complete AZFc microdeletion accounted for 63.2% (198/313), and of the 198 patients with AZFc microdeletion, 69.7% (138/198) showed azoospermia. Forty-six (33.3%) of the azoospermic patients had sperm in their semen after AI administration. Testosterone (T) and testosterone-to-estradiol ratio (T/E_2) levels were higher in group A than those in group B after treatment, and the differences were significant (T, $P = 0.038$; T/E_2, $P = 0.004$). Paired *t*-test demonstrated that the change of T levels before and after treatment was statistically significant ($P = 0.003$). The increased E_2 and T/E_2 ratio levels before and after treatment were not statistically significant ($P = 0.057$ and 0.080), but they were close to the threshold value ($P = 0.05$). *Conclusions*. Patients with AZFc microdeletion accounted for the largest proportion of male infertility caused by Y chromosome microdeletions. AIs can promote spermatogenesis in azoospermic patients with AZFc microdeletion, and sperm could be found in the semen of some patients after AI administration. T and T/E_2 levels after AI treatment could be used as biomarkers to distinguish azoospermic patients with AZFc microdeletion who responded better to AIs from those who did not respond well.

1. Introduction

Infertility affects approximately 15% of couples trying to conceive worldwide, and half of the infertile cases resulted from male factors [1]. Most infertile males are diagnosed with spermatogenic failure [2]. Y chromosome microdeletions are the most common genetic etiology for spermatogenic failure [3], accounting for 2%–10% in infertile males with severe oligozoospermia (<5 million sperm/ml ejaculate) or azoospermia [4, 5]. The azoospermia factor (AZF) regions on the Y chromosome contain several genes that are essential for spermatogenesis [2]. The most prevalent AZF deletion type is the AZFc microdeletion (approximately 80%) [6].

Infertile men with AZFc microdeletion have clinical phenotypes that range from oligozoospermia to azoospermia. Microdissection testicular sperm extraction (micro-TESE) combined with intracytoplasmic sperm injection (ICSI) has been used to help many couples who are affected by

azoospermia to produce their reproductive offspring. However, these technologies are expensive and have their own limitations [7, 8]. For example, men with AZFc microdeletion resulting in infertility may produce sons by intracytoplasmic sperm injection, but there is a risk of transmission of the deletion and infertility [9]. Preimplantation genetic diagnosis before ICSI can avoid the vertical transmission of AZFc microdeletion [10].

Aromatase inhibitors (AIs) can increase endogenous testosterone production [11], and high testosterone levels in testis are indispensable for spermatogenesis [12], a complicated process that is precisely regulated by the hypothalamic-pituitary-gonadal axis [13, 14]. Spermatogenic disorder can be alleviated by AIs, which regulate the hypothalamic-pituitary-gonadal axis by regulating testosterone levels in patients with increased estradiol levels [11]. AI administration can effectively increase spontaneous pregnancy by improving sperm parameters in men with idiopathic oligozoospermia and can support the reappearance of sperm in the ejaculate of patients with nonobstructive azoospermia [15, 16]. Endocrine hormone disorders have been reported in patients with AZFc microdeletion [17]. However, the efficacy of AIs in patients with azoospermia caused by AZFc microdeletion has not been reported so far. In the present study, we retrospectively analyzed the clinical features and rate of reappearance of sperm in the semen of men with azoospermia and AZFc microdeletion and evaluated the correlation between the reappearance of sperm in the semen and clinical evaluation parameters.

2. Materials and Methods

2.1. Subjects. Patients with AZFc microdeletion who were admitted to Shanghai General Hospital from March 2015 to March 2021 were selected for this study. We screened 3364 patients with AZFc microdeletion who were infertile, and 313 (9.30%) of them were enrolled in the present study. This study was approved by the Ethics Committee of Shanghai General Hospital (ethics approval number: 2020SQ199).

2.2. Inclusion Criteria. Azoospermic patients with complete AZFc microdeletion were verified by qPCR examination at Shanghai General Hospital. According to the European Academy of Andrology (EAA) criteria for molecular diagnosis of Y chromosomal microdeletions, eight loci were investigated for sY14 (SRY), ZFX/ZFY, sY84, sY86, sY127, sY134, sY254, and sY255, of which sY254 and sY255 were both absent with regard to the AZFc microdeletion [4].

2.3. Exclusion Criteria. Other abnormal genotypes related to spermatogenic disorders were excluded by karyotype analysis. Patients diagnosed with partial AZFc microdeletion were excluded.

2.4. AI Treatment. Azoospermic patients with complete AZFc microdeletion were treated with AIs (letrozole 2.5 mg/day or anastrozole 1 mg/day, 3–6 months, orally).

2.5. Data Collection and Analysis. After AI treatment, the patients with azoospermia were divided into two groups: those who had sperm in their semen (group A) and those without sperm in their semen (group B). All the patients were further divided into three subgroups based on their sperm analysis: oligozoospermia, cryptozoospermia, and azoospermia. Clinical parameters (height, weight, karyotype analysis, Y chromosome microdeletion detection, volume of testes, and history of mumps orchitis, varicocele, cryptorchidism, or chemical chronic irritation) and hormone levels (prolactin (PRL), testosterone (T), follicle-stimulating hormone (FSH), and luteinizing hormone (LH)) before and after AI administration were collected.

2.6. Statistics. An independent t-test was used to compare differences of continuous data between groups A and B. The paired t-test was used to compare differences in the assessed parameters of the same patient before and after AI administration. All the results are expressed as mean ± SD. The chi-square test or Fisher's exact test was used for binomial distribution data, and all the tests were two-sided. $P < 0.05$ was considered statistically significant. The statistical analyses were performed using SPSS 26.0.

3. Results

3.1. Heterogeneity of Clinical Phenotypes in Patients with AZFc Microdeletion. A total of 3364 infertile men were enrolled in this study (Figure 1). The rate of Y chromosome AZF microdeletions was 9.15% (308/3364), among which patients with complete AZFc microdeletion accounted for 64.3% (198/308). Among the 198 patients with AZFc microdeletion, 27 (13.6%) showed oligozoospermia, 33 (16.7%) showed cryptozoospermia, and 138 (69.7%) showed azoospermia. All patients with azoospermia were treated with AIs, and 46 (33.3%) of them had sperm in their semen after AI administration.

3.2. Patients with Azoospermia Who Had Sperm in Their Semen after AI Administration. A total of 46 patients (33.3%, 46/138) with azoospermia were found to have sperm in their semen after AI administration (Table 1). The left testicular volume of patients in group A was significantly higher than that of patients in group B ($P = 0.032$, Table 1) before AI administration. There were no significant differences in other clinical parameters before AI administration between patients in the two groups (Table 1).

We retrospectively analyzed the hormone levels of azoospermic patients with AZFc microdeletion and after AI treatment (Table 2). LH, T, and T/E_2 levels were higher in group A than those in group B after treatment, and the differences were significant (LH, $P = 0.030$; T, $P = 0.038$; T/E2, $P = 0.004$). The FSH level was higher in group A than that in group B after treatment, but the difference was not statistically significant ($P = 0.327$). There were no significant differences in other clinical parameters after AI administration between the patients in the two groups (Table 2).

A paired sample t-test was used to compare the changed levels of sexual hormones in patients with sperm in the semen after AI treatment (Table 3). Paired t-test demonstrated that the change in T levels before and after treatment

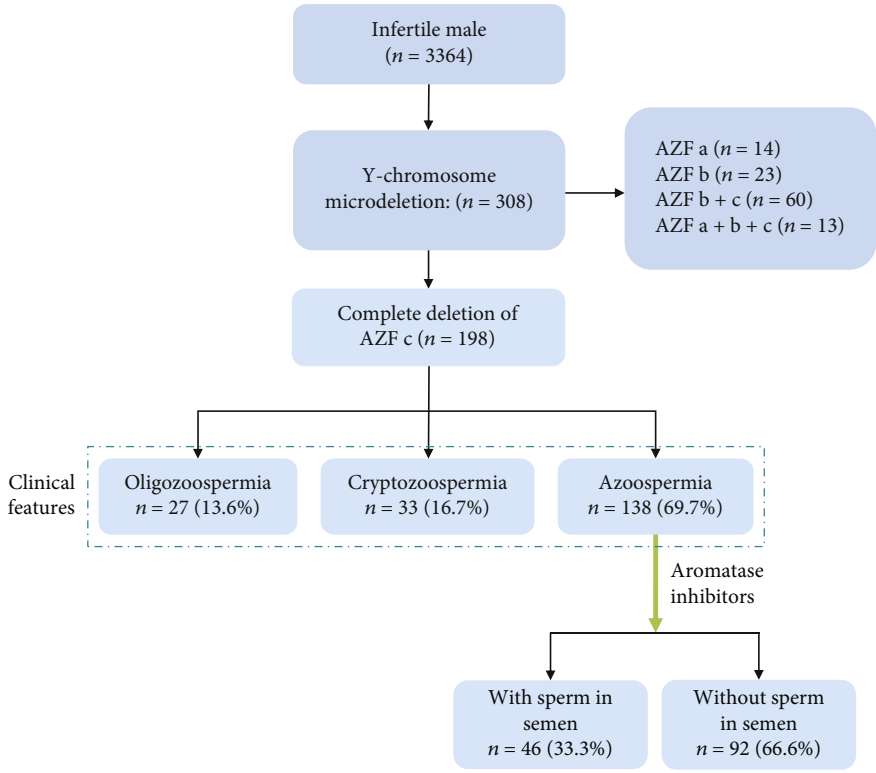

Figure 1: Screening of enrolled patients with Y chromosome microdeletions.

Table 1: Clinical parameters of patients with azoospermia and AZFc microdeletion before aromatase inhibitor administration.

Clinical parameters	Group A ($n = 46$)			Group B ($n = 92$)			P value
	Min	Max	Mean ± S	Min	Max	Mean ± S	
Age	23	45	29.95 ± 3.69	22	43	31.04 ± 5.32	0.113
BMI	19.37	30.56	23.83 ± 5.54	17.04	24.67	22.13 ± 2.54	0.688
Testicular volume right (ml)	2	20	11.46 ± 2.59	6	15	10.28 ± 2.86	0.065
Testicular volume left (ml)	2	20	11.41 ± 2.69	6	15	10.02 ± 2.79	0.032
FSH_b (mIU/ml)	1.70	78.79	14.90 ± 14.92	3.07	56.89	18.30 ± 10.42	0.155
LH_b (mIU/ml)	1.24	28.08	7.24 ± 5.54	0.85	66.31	8.17 ± 7.86	0.5
PRL_b (ng/ml)	0.54	36.66	12.89 ± 7.81	0.39	36.31	12.31 ± 7.99	0.728
E_2_b (pg/ml)	1.59	142.47	34.68 ± 26.95	4.00	95.00	30.98 ± 18.27	0.231
T_b (ng/ml)	0.62	9.73	3.83 ± 1.95	0.23	15.45	4.03 ± 2.62	0.671
T/E_2_b (ng/pg)	0.02	6.12	0.39 ± 1.13	0.01	0.77	0.17 ± 0.15	0.142
Duration of infertility (year)	1	8	2.50 ± 1.71	1	10	2.93 ± 1.96	0.298

Group A: patients with sperm detected in the semen after AI administration; Group B: patients without sperm detected in the semen after AI administration; E_2_b: estradiol of patients with azoospermia and AZFc microdeletion before AI administration; T_b: total testosterone of patients with azoospermia and AZFc microdeletion before AI administration.

was statistically significant ($P = 0.003$), but the correlation test showed that the correlation of testosterone levels before and after treatment in patients was not obvious ($r = 0.117$, $P = 0.614$). The increased E_2 and T/E_2 ratio levels before and after treatment were not statistically significant ($P = 0.057$ and 0.080), but they were close to the threshold value ($P = 0.05$), which may be due to the small sample size (13/46 and 14/46).

3.3. Effects of Chronic Inflammatory Stimulation on the Outcomes of AI Administration. The proportion of patients with a history of chronic inflammation was lower in group

Table 2: Sexual hormone levels of patients with azoospermia and AZFc microdeletion after aromatase inhibitor administration.

	Group A ($n = 46$)			Group B ($n = 92$)			P value
	Min	Max	Mean ± S	Min	Max	Mean ± S	
FSH_a (mIU/ml)	1.92	57.48	22.64 ± 14.57	2.06	68.50	21.41 ± 13.18	0.327
LH_a (mIU/ml)	2.12	42.99	13.90 ± 11.51	0.77	28.71	10.98 ± 6.98	0.030
PRL_a (ng/ml)	0.33	29.79	10.05 ± 8.35	0.07	234.00	20.19 ± 38.60	0.178
E_2_a (pg/ml)	5.00	90.23	22.80 ± 20.52	3.81	125.40	29.30 ± 24.05	0.694
T_a (ng/ml)	0.29	13.89	6.69 ± 3.60	0.96	11.43	5.52 ± 2.69	0.038
T/E_2_a (ng/pg)	0.02	1.79	0.55 ± 0.53	0.02	1.45	0.31 ± 0.29	0.004

Group A: patients with sperm detected in the semen after AI administration; Group B: patients without sperm detected in the semen after AI administration; E_2_a: estradiol of patients with azoospermia and AZFc microdeletion after AI administration; T_a: total testosterone of patients with azoospermia and AZFc microdeletion after AI administration.

Table 3: Changed levels of sexual hormones before and after aromatase inhibitor (AI) treatment of patients in group A.

The changed sexual hormone levels	Correlation test		Paired t-test	
	r value	P value	Mean ± S	P value
T/E_2_a-T/E2_b	0.002	0.996	0.19 ± 0.41	0.080
T_a-T_b	0.117	0.614	2.07 ± 3.27	0.003
FSH_a-FSH_b	0.048	0.831	4.07 ± 17.18	0.331
LH_a-LH_b	-0.204	0.363	4.23 ± 10.55	0.108
E_2_a-E_2_b	-0.332	0.267	−4.04 ± 31.56	0.057
PRL_a-PRL_b	0.300	0.259	4.25 ± 35.31	0.198

_a: after AI administration; _b: before AI administration; r value: Pearson's correlation coefficient.

Table 4: History of chronic inflammation in patients with azoospermia and AZFc microdeletion.

	Group A ($n = 46$)	Group B ($n = 92$)	P value
With chronic inflammation	7 (15.2%)	20 (21.7%)	0.495
Mumps orchitis	3 (6.5%)	6 (6.5%)	
Cryptorchidism	0	2 (2.2%)	
Varicocele	4 (8.7%)	10 (10.9%)	
Chemical chronic irritation	0	2 (2.2%)	
Without inflammation	39 (84.8%)	72 (78.3%)	
Total	46	92	

Group A: patients with sperm detected in the semen after AI administration; Group B: patients without sperm detected in the semen after AI administration.

A (8.7%) than it was in group B (15.3%), but the difference was not statistically significant ($P = 0.495$, Table 4). The rates of mumps orchitis were the same in group A (6.5%) and group B (6.5%), and the rates of cryptorchidism and varicocele and a history of chemical exposure were lower in group A than they were in group B after AI administration (0% vs. 2.2%, 4% vs. 10.9%, and 0% vs. 2.2%, respectively).

4. Discussion

In the present study, we found that AZFc microdeletion accounted for the largest proportion of Y chromosome microdeletions, which is consistent with the prevalence reported previously [5]. Spermatogenic failure, clinically known as oligozoospermia, cryptozoospermia, or azoospermia, can result from the AZFc microdeletion. But some patients' testicles still go through the entire spermatogenic process [18]. It is possible that patients with AZFc deletions respond effectively to AI therapy because spermatogenic tissue is still present in their testicles.

Our research shows that 33.3% of azoospermic individuals with AZFc microdeletion were able to have sperm in their semen through the advent of AIs. After receiving AI treatments, patients in group A had significantly higher T and T/E_2 levels than those in group B, which is consistent with the earlier research showing that AI treatment is beneficial for patients with spermatogenic failure who have lower T/E_2 ratios and T levels [15, 19]. After using AIs, T levels are anticipated to rise and E_2 levels to fall since T to E_2 conversion depends on aromatase, which can be inhibited by AIs [20]. Paired t-test results in azoospermic patients who had sperm in the semen following AI treatment demonstrated that the altered T levels were statistically significant, supporting the efficacy of AI. Collectively, our results indicated that T and T/E_2 levels after treatment could be used as biomarkers to distinguish patients with azoospermia and AZFc

microdeletion who had a better response to AIs from patients who had no response to AIs.

We also investigated the difference in the proportion of azoospermic patients after AI treatment with and without a history of chronic inflammation. The proportion of patients with a history of chronic inflammation was lower in patients with sperm in their semen than it was in patients without sperm in their semen. However, the difference was not statistically significant, possibly due to the insufficient sample size. Varicocele and cryptorchidism have been reported as complications of AZFc microdeletion [21, 22], but the treatment outcomes have not been investigated. Our results indicated that chronic inflammation such as varicocele and cryptorchidism can adversely affect the outcome of AI treatment in patients with AZFc microdeletion. However, large sample multicenter clinical studies are needed to confirm these findings.

A limitation of this study is the lack of a blank control group that received placebo administration. The Urologic Medical Center (Shanghai General Hospital) is in the process of applying for clinical trials that will include more patients and set more reasonable groups to study the efficacy of AIs on patients with azoospermia.

5. Conclusions

AZFc microdeletion accounts for the largest proportion of male infertility caused by the Y chromosome. AIs can promote spermatogenesis in azoospermic patients with AZFc microdeletion, and sperm was found in the semen of some patients after AI administration. T and T/E_2 levels after treatment could be used as biomarkers to distinguish azoospermic patients with AZFc microdeletion who responded better to AIs from those who did not respond to AIs.

Data Availability

Data is available upon reasonable request to the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

L.Z. and Y.C.C. designed and conceived the study. O.N.J., Z.J.X., L.S.W., and S.Y.F. collected and analyzed the clinical data. O.N.J. interpreted the data and wrote the paper. L.Z. and Y.C.C read and amended the manuscript. T.R.H., L.P., Z.E.L., Z.Y.X., B.H.W., Z.J.P., and H.Y.H helped in collecting the clinical data. All authors reviewed and approved the final manuscript. Ningjing Ou, Yifan Sun, Jianxiong Zhang, and Shiwei Liu have contributed equally to this work.

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References

[1] C. L. R. Barratt, L. Björndahl, C. J. De Jonge et al., "The diagnosis of male infertility: an analysis of the evidence to support the development of global WHO guidance-challenges and future research opportunities," *Human Reproduction Update*, vol. 23, no. 6, pp. 660–680, 2017.

[2] C. Krausz and E. Casamonti, "Spermatogenic failure and the Y chromosome," *Human Genetics*, vol. 136, no. 5, pp. 637–655, 2017.

[3] T. Kuroda-Kawaguchi, H. Skaletsky, L. G. Brown et al., "The _AZFc_ region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men," *Nature Genetics*, vol. 29, no. 3, pp. 279–286, 2001.

[4] C. Krausz, L. Hoefsloot, M. Simoni, F. Tüttelmann, European Academy of Andrology, and European Molecular Genetics Quality Network, "EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013," *Andrology*, vol. 2, no. 1, pp. 5–19, 2014.

[5] M. Simoni, F. Tüttelmann, J. Gromoll, and E. Nieschlag, "Clinical consequences of microdeletions of the Y chromosome: the extended Munster experience," *Reproductive Biomedicine Online*, vol. 16, no. 2, pp. 289–303, 2008.

[6] P. Hallast, L. Kibena, M. Punab et al., "A common 1.6 mb Y-chromosomal inversion predisposes to subsequent deletions and severe spermatogenic failure in humans," *eLife*, vol. 10, 2021.

[7] C. Niederberger, A. Pellicer, J. Cohen et al., "Forty years of IVF," *Fertility and Sterility*, vol. 110, no. 2, pp. 185–324.e5, 2018.

[8] P. N. Schlegel, M. Sigman, B. Collura et al., "Diagnosis and treatment of infertility in men: AUA/ASRM guideline part II," *The Journal of Urology*, vol. 205, no. 1, pp. 44–51, 2021.

[9] D. C. Page, S. Silber, and L. G. Brown, "Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility," *Human Reproduction*, vol. 14, no. 7, pp. 1722–1726, 1999.

[10] X. H. Liu, J. Qiao, R. Li, L. Y. Yan, and L. X. Chen, "Y chromosome AZFc microdeletion may not affect the outcomes of ICSI for infertile males with fresh ejaculated sperm," *Journal of Assisted Reproduction and Genetics*, vol. 30, no. 6, pp. 813–819, 2013.

[11] C. P. Pavlovich, P. King, M. Goldstein, and P. N. Schlegel, "Evidence of a treatable endocrinopathy in infertile men," *The Journal of Urology*, vol. 165, no. 3, pp. 837–841, 2001.

[12] J. P. Jarow and B. R. Zirkin, "The androgen microenvironment of the human testis and hormonal control of spermatogenesis," *Annals of the New York Academy of Sciences*, vol. 1061, no. 1, pp. 208–220, 2005.

[13] F. T. Neto, P. V. Bach, B. B. Najari, P. S. Li, and M. Goldstein, "Spermatogenesis in humans and its affecting factors," *Seminars in Cell & Developmental Biology*, vol. 59, pp. 10–26, 2016.

[14] L. Zhao, C. Yao, X. Xing et al., "Single-cell analysis of developing and azoospermia human testicles reveals central role of Sertoli cells," *Nature Communications*, vol. 11, no. 1, p. 5683, 2020.

[15] L. Kooshesh, S. Bahmanpour, S. Zeighami, and M. H. Nasr-Esfahani, "Effect of letrozole on sperm parameters, chromatin status and ROS level in idiopathic oligo/astheno/teratozoospermia," *Reproductive Biology and Endocrinology*, vol. 18, no. 1, p. 47, 2020.

[16] C. Yang, P. Li, and Z. Li, "Clinical application of aromatase inhibitors to treat male infertility," *Human Reproduction Update*, vol. 28, no. 1, pp. 30–50, 2021.

[17] I. S. Huang, W. J. Chen, L. H. Li, R. E. Brannigan, and W. J. Huang, "The predictive factors of successful sperm retrieval for men with Y chromosome AZFc microdeletion," *Journal of Assisted Reproduction and Genetics*, vol. 39, no. 10, pp. 2395–2401, 2022.

[18] C. Y. Deng, Z. Zhang, W. H. Tang, and H. Jiang, "Microdeletions and vertical transmission of the Y-chromosome azoospermia factor region," *Asian Journal of Andrology*, vol. 25, no. 1, pp. 5–12, 2023.

[19] B. Saylam, O. Efesoy, and S. Cayan, "The effect of aromatase inhibitor letrozole on body mass index, serum hormones, and sperm parameters in infertile men," *Fertility and Sterility*, vol. 95, no. 2, pp. 809–811, 2011.

[20] G. Séralini and S. Moslemi, "Aromatase inhibitors: past, present and future," *Molecular and Cellular Endocrinology*, vol. 178, no. 1-2, pp. 117–131, 2001.

[21] R. Dada, N. P. Gupta, and K. Kucheria, "AZF microdeletions associated with idiopathic and non-idiopathic cases with cryptorchidism and varicocele," *Asian Journal of Andrology*, vol. 4, no. 4, pp. 259–263, 2002.

[22] E. P. de Sousa Filho, D. M. Christofolini, C. P. Barbosa, S. Glina, and B. Bianco, "Y chromosome microdeletions and varicocele as aetiological factors of male infertility: a cross-sectional study," *Andrologia*, vol. 50, no. 3, p. e12938, 2018.