ORIGINAL ARTICLE

# Roles of bovine *Waddlia chondrophila* and *Chlamydia trachomatis* in human preterm birth

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## Abstract

*Waddlia chondrophila* and *Chlamydia trachomatis* are intracellular bacteria associated with human miscarriage. We investigated their role in human preterm birth. Whereas presence of *Chlamydia trachomatis* DNA in genital tract was associated with human preterm birth, *Waddlia* was not, despite being present in women's genital tracts.



**Keywords:** Adverse pregnancy outcome, *Chlamydia*-like, genital tract infection, intracellular bacteria, preterm birth
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## Introduction

In 2010, approximately 15 million babies were born preterm worldwide, and more than one million died due to complications from preterm births (PTB). Neonates that survive PTB exhibit an increased risk of neurodevelopmental impairments and respiratory complications [1]. The proportion of spontaneous PTB attributed to infection is approximately 50% [2]. However, a pathogen is identified in only one third of the cases, despite evidence of infection. Obligate intracellular bacteria, which do not grow on media used routinely to isolate human pathogens from clinical samples, might represent possible agents of PTB.

*Chlamydia trachomatis,* an obligate intracellular bacterium, is considered the world's most common sexually transmitted bacterial pathogen. *Waddlia chondrophila* is another member of the Chlamydiales order that has been shown to cause abortions in bovines [3]. Both of these intracellular bacteria have also been implicated in human adverse pregnancy outcomes [4–7]. In addition, *C. trachomatis* is known to cause premature rupture of the membranes and premature uterine activity, and growing evidence suggests a role for *C. trachomatis* in PTB [8]. In this study, we investigated the role of *Waddlia* and *Chlamydia* as emerging agents of PTB. We studied 407 women with PTBs or uneventful term pregnancies attending the University Hospital of Lausanne, Lausanne, Switzerland. In addition to serology, we also performed PCR to detect *Waddlia* and *Chlamydia* in the placenta and vaginal samples taken from these women, as well as histology on the placenta.

From 2006 to 2009, 407 women were enrolled into this study at the obstetrical ward of the University Hospital of Lausanne. The PTB group ($n$ = 146) included women who spontaneously delivered before 37 weeks' gestation. The control group ($n$ = 261) included women attending a labour ward with uneventful term pregnancies and no history of miscarriages, stillbirths or preterm labour. We compared demographic data and risk factors of patients with and without PTB or *C. trachomatis* infection by the Pearson $\chi^2$ test (or the Fisher exact test when indicated) for categorical variables. For continuous variables, medians were compared by the Wilcoxon-Mann-Whitney test. Multivariable analyses were performed to control for covariates. Statistical analyses were performed using the Stata software, version 13.0 (StataCorp, College Station, TX, USA).



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Only positive urine cultures, gestational and maternal age were significantly different between control and PTB groups (Table 1). Other infectious causes were investigated in the PTB group, showing that a positive culture was found in the vagina, maternal or fetal side of the placenta in 32%, 37% and 17% of the PTB patients, respectively (Supplementary Table 1). Among all these bacterial species, genital mycoplasma and *Gardnerella vaginalis,* which have been previously associated with PTB [9], were recovered in some subjects from the vagina only ($n$ = 25 and 3, respectively), never from the placenta.

Sera were tested for antibodies directed against *C. trachomatis* and *W. chondrophila*, respectively, by using the MOMP-R, CT pELISA (R-biopharm, Darmstadt, Germany) [10] and *Waddlia*-specific immunofluorescence as described elsewhere [6]. Isolated IgG mainly reflects past or chronic infection, whereas IgM and/or IgA reflect acute infection. Briefly, *W. chondrophila* strain ATCC VR-1470 was used as antigen, whereas Fluoline G or M (bioMérieux, Marcy l'Étoile, France) were used as secondary antibodies. An antibody titre of ≥1/64 for IgG and ≥1/32 for IgM were considered as positive, respectively [6]. There was no difference between control and PTB groups in terms of anti-*Chlamydia* IgG and IgA and anti-*Waddlia* IgG and IgM titres (Table 1). A total of 54 patients tested positive only for *Waddlia* IgG and 26 only for *Chlamydia* IgG, indicating the absence of serologic cross-reaction between both pathogens. Only six patients tested positive for both *Waddlia* and *Chlamydia* IgG (p 0.446).

*C. trachomatis* IgG seropositivity (Table 2) was associated with civil status (divorced vs. married, odds ratio (OR) 7.85; 95% confidence interval (CI) 2.61–23.62), education (OR 0.28; 95% CI 0.10–0.81) and number of previous sexual partners (>6 vs. 1: OR 13.12, 95% CI 1.53–112.32; "not answered" vs. 1: OR 13.55, 95% CI 1.76–104.09). Patients who used condoms as a previous contraceptive method show less *C. trachomatis* positive serologies, although this was not statistically significant. Of note, only six (55%) and one (9%) of the 11 patients positive for *C. trachomatis* DNA were also positive for *C. trachomatis* IgG and IgA, respectively. However, *C. trachomatis* IgG-positive patients exhibited significantly more histologic chorioamnionitis (50%) than *C. trachomatis* IgG-negative patients (28.3%, p 0.015).

**TABLE 1. Characteristics of patients according to term history**

| Characteristic | Control (n = 261) | PTB (n = 146) | p |
|---|---|---|---|
| Gestational age at birth, weeks, ±SD | 39.6 ± 1.1 | 32.6 ± 3.3 | <0.001 |
| Age, years, ±SD | 31.5 ± 5.0 | 32.4 ± 5.9 | 0.057 |
| <35 years | 194 (74.3%) | 94 (64.4%) | 0.041 |
| ≥35 years | 67 (25.7%) | 52 (35.6%) | |
| Parity, ±SD | 0.5 ± 0.8 | 0.5 ± 0.8 | 0.950 |
| 0 | 160 (61.3%) | 95 (65.1%) | 0.228 |
| 1 | 72 (27.6%) | 30 (20.6%) | |
| >1 | 29 (11.1%) | 21 (14.4%) | |
| Origin | | | |
| European | 217 (83.1%) | 113 (77.4%) | 0.156 |
| Non-European | 44 (16.9%) | 33 (22.6%) | |
| Civil status | | | |
| Married | 201 (77.0%) | 109 (74.7%) | 0.739 |
| Single | 49 (18.8%) | 32 (21.9%) | |
| Divorced | 11 (4.2%) | 5 (3.4%) | |
| Education | | | |
| Nonuniversity studies | 170 (65.1%) | 106 (72.6%) | 0.150 |
| University studies | 91 (34.9%) | 40 (27.4%) | |
| No. of lifelong sexual partners | | | |
| 1 | 58 (22.2%) | 37 (25.3%) | 0.393 |
| 2–3 | 43 (16.5%) | 29 (19.9%) | |
| 4–6 | 45 (17.2%) | 19 (13.0%) | |
| >6 | 36 (13.8%) | 13 (8.9%) | |
| Not answered | 79 (30.3%) | 48 (32.9%) | |
| Condom as previous contraceptive method | 69 (26.4%) | 33 (22.6%) | 0.407 |
| Smoking status | | | |
| Nonsmoker | 224 (85.8%) | 129 (88.4%) | 0.543 |
| Smoker | 37 (14.2%) | 17 (11.6%) | |
| Pets at home | 82 (31.4%) | 39 (26.7%) | 0.366 |
| Vegetarian | 5 (1.9%) | 5 (3.4%) | 0.341 |
| *Chlamydia trachomatis* serology | | | |
| IgG positive | 19 (7.3%) | 13 (8.9%) | 0.569 |
| IgA positive | 10 (3.8%) | 9 (6.2%) | 0.330 |
| Both IgG and IgA positive | 7 (2.7%) | 7 (4.8%) | 0.270 |
| *C. trachomatis* PCR | | | |
| Cervicovaginal swab | 2 (0.7%) | 7 (4.8%) | 0.012 |
| Placenta | 2 (0.7%) | 7 (4.8%) | 0.012 |
| At least one PCR positive | 2 (0.7%) | 9 (6.2%) | 0.002 |
| *Waddlia* serology | | | |
| Total Ig ≥1/64 | 47 (18.0%) | 31 (21.2%) | 0.428 |
| IgG ≥1/64 | 38 (14.6%) | 22 (15.1%) | 0.889 |
| IgM ≥1/16 | 9 (3.5%) | 11 (7.5%) | 0.092 |
| *Waddlia* PCR | | | |
| Cervico-vaginal swab | 11 (4.2%) | 10 (6.9%) | 0.252 |
| Placenta | 11 (4.2%) | 4 (2.7%) | 0.587 |
| Other infections | | | |
| Positive urine culture | 7 (2.7%) | 38 (26%) | <0.001 |
| *Streptococcus agalactiae* | 45 (18.0%) | 22 (17.5%) | 1.000 |
| *Brucella abortus* | 19 (7.3%) | 11 (7.5%) | 1.000 |
| *Parachlamydia acanthamoebae* | 2 (0.8%) | 0 (0%) | 0.539 |
| *Simkania negevensis* | 3 (1.2%) | 1 (0.7%) | 1.000 |

PTB, preterm birth.

**TABLE 2. Characteristics of patients according to *Chlamydia trachomatis* serologic status**

| Characteristic | IgG negative (n = 375, 92.1%) | IgG positive (n = 32, 7.9%) | p |
|---|---|---|---|
| Age, years, ±SD | 31.9 ± 5.3 | 30.9 ± 6.5 | 0.298 |
| <35 years | 263 (91.3%) | 25 (8.7%) | 0.421 |
| ≥35 years | 112 (94.1%) | 7 (5.9%) | |
| Parity, ±SD | 0.5 ± 0.8 | 0.5 ± 0.7 | 0.997 |
| 0 | 233 (91.4%) | 22 (8.6%) | 0.38 |
| 1 | 97 (95.1%) | 5 (4.9%) | |
| >1 | 45 (90%) | 5 (10.0%) | |
| Origin | | | |
| European | 307 (93.0%) | 23 (7.0%) | 0.164 |
| Non-European | 68 (88.3%) | 9 (11.7%) | |
| Civil status | | | |
| Married | 288 (92.9%) | 22 (7.1%) | >0.001 |
| Single | 77 (95.1%) | 4 (4.9%) | |
| Divorced | 10 (62.5%) | 6 (37.5%) | |
| Education | | | |
| Nonuniversity studies | 248 (89.9%) | 28 (10.1%) | 0.017 |
| University studies | 127 (97.0%) | 4 (3.1%) | |
| No. of lifelong sexual partners | | | |
| 1 | 94 (99.0%) | 1 (1.0%) | 0.006 |
| 2–3 | 66 (91.7%) | 6 (8.3%) | |
| 4–6 | 61 (95.3%) | 3 (4.7%) | |
| >6 | 43 (87.8%) | 6 (12.2%) | |
| Not answered | 111 (87.4%) | 16 (12.6%) | |
| Place of residence | | | |
| Rural | 126 (91.3%) | 12 (8.7%) | 0.699 |
| City | 249 (92.6%) | 20 (7.4%) | |
| Condom as previous contraceptive method | | | |
| No | 277 (90.8%) | 28 (9.2%) | 0.094 |
| Yes | 98 (96.1%) | 4 (3.9%) | |
| Smoking status | | | |
| Nonsmoker | 209 (95.0%) | 11 (5.0%) | 0.114 |
| Smoker | 48 (88.9%) | 6 (11.1%) | |

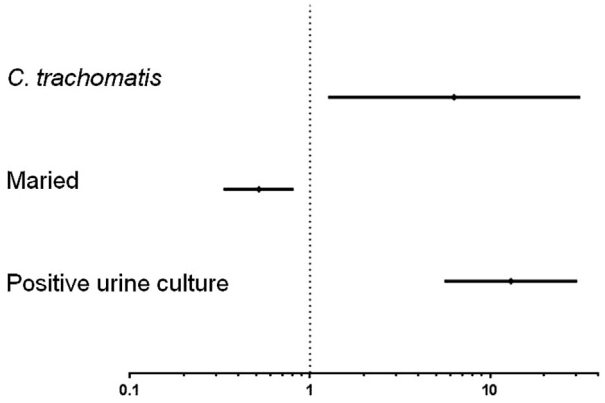


**FIG. 1.** Stepwise logistic regression showing independent association of positive *Chlamydia trachomatis* PCR with preterm birth.

*Waddlia* seropositivity was not associated with age, number of lifelong sexual partners, place of residence (rural vs. urban), smoking, pet ownership or meat consumption (data not shown). Interestingly, 4.9% of women of European heritage vs. 14.3% of those of non-European heritage had positive *Waddlia* IgG serologies of ≥1/256 (p 0.008). This correlation was also observed for *Waddlia* IgM of ≥1/32 (3.6% of European vs. 10.4% of non-European, p 0.034). Among non-European women, the highest *Waddlia* IgG titres (≥1/128 or ≥1/256) were more frequent for black ethnicity (20% and 14.3%, respectively). When total immunoglobulin (Ig ≥1/64) against *Waddlia* was considered, condoms as a previous contraceptive method protected against *Waddlia* infection (12.1% condom users vs. 21.4% of the non-condom users exhibited anti-*Waddlia* Ig reactivity, p 0.041).

After DNA extraction with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), all vaginal swabs and placenta samples were screened for *C. trachomatis* DNA by a TaqMan real-time PCR targeting the cryptic plasmid of *C. trachomatis*, as described earlier [11]. Samples were also tested by a 16S rRNA *Waddlia*-specific real-time PCR, as described previously [12]. No PCR inhibition was observed for either pathogen. Vaginal and placental samples from the PTB group were significantly more often *C. trachomatis* DNA positive than the control group (p 0.002, unadjusted OR 8.51; 95% CI 1.81–39.93). This association reminded significant even after adjustment for maternal age, origin, civil status, education, number of sexual partners and positive urine cultures (OR 7.93; 95% CI 1.34–46.76). Stepwise logistic regression allowed us to confirm that the presence of *C. trachomatis* DNA is an independent factor associated with PTB (Fig. 1). There was no difference between the control and PTB groups regarding *Waddlia* PCR results. Thirty-four patients were positive only for *W. chondrophila,* whereas ten other subjects were positive only for *C. trachomatis* by PCR. Only one patient was positive for both, demonstrating the absence of cross-amplification of these two obligate intracellular bacteria. Presence of *Waddlia* DNA was demonstrated in the genital tract or placenta of 13 PTB subjects (Table 3). Seven of them had more than one miscarriage in their medical history. Of these 13 patients, four exhibited both a positive

**TABLE 3. Clinical history, serology, PCR and placental histology of preterm patients with samples positive for *Waddlia* by real-time PCR**

| Patient no. | Maternal age, years | No. pregnancies | Parity | Gestational age at birth, weeks | Birth weight, g | Country of origin | *Waddlia* IgG | *Waddlia* IgM | *Waddlia* PCR positive | Placental histology | Other possible etiologies[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | 37 | 2 | 0 | 35.3 | 2520 | Switzerland | — | — | P | Calcifications | |
| 28 | 35 | 10 | 4 | 31.5 | 1870 | Angola | — | — | P and VS | Subchorial fibrin, necrosis, lymphocytes in chorion and amnion, vasculitis | |
| 45 | 27 | 1 | 0 | 34.1 | 2340 | Switzerland | — | — | VS | — | |
| 66 | 29 | 1 | 0 | 25.6 | 890 | Portugal | — | — | VS | Subchorial fibrin, lymphocytes in chorion and amnion, oedema | |
| 133 | 40 | 2 | 1 | 30.5 | 1270 | Togo | 1/128 | — | VS | Villous oedema | |
| 185 | 33 | 2 | 0 | 35.1 | 2060 | Switzerland | — | — | VS | Villous oedema | |
| 223 | 32 | 2 | 0 | 36.3 | 2370 | Portugal | — | — | VS | — | CT IgG and IgA positive[b] |
| 261 | 39 | 4 | 0 | 34.4 | 2540 | Italy | 1/128 | — | P | Decidual fibrin | |
| 283 | 34 | 4 | 1 | 36.4 | 2560 | Congo | 1/256 | — | VS | Subchorial fibrin | |
| 314 | 36 | 1 | 0 | 31.3 | 1310 | Switzerland | — | — | VS | — | |
| 351 | 33 | 2 | 0 | 35.3 | 2280 | Italy | 1/128 | 1/32 | VS | Marginal hemorrhage | |
| 476 | 35 | 2 | 1 | 30 | 1530 | Italy | — | — | VS | Lymphocytes in decidua | |
| 572 | 26 | 1 | 0 | 35 | 1920 | Switzerland | — | — | P and U | Intervillous hemorrhage | CT PCR positive placenta[c] |

Haematoxylin and eosin–stained histologic sections of all placenta specimens were examined for the type and degree of placentitis, endometritis and/or vasculitis by a pedopathologist.
P, placenta; VS, vaginal swab; U, urine; CT, *C. trachomatis.*
[a]All patients were also tested for *C. trachomatis, Brucella abortus, Streptococcus agalactiae, Parachlamydia acanthamoebae* and *Simkania negevensis.*
[b]Positive serology for *C. trachomatis* observed in this case reflects a possible coinfection because there is no serologic cross-reaction between *C. trachomatis* and *Waddlia chondrophila,* and because the *W. chondrophila* serology was negative.
[c]*C. trachomatis* positive PCR reflects a likely coinfection because there is no cross-amplification with the PCRs we used.

*Waddlia* serology and presence of *Waddlia* DNA. Two had presence of *Waddlia* in multiple samples, including vaginal swab, urine and placenta. Only two of these 13 patients had either a positive *C. trachomatis* serology or PCR. Ten of these 13 patients had abnormal placenta histologies.

## Discussion

Both *C. trachomatis* and *W. chondrophila* have been implicated in adverse pregnancy outcomes [4,5,7,8]. However, there have been contradictory findings pertaining to the role of *C. trachomatis* infection in PTB. A few small studies have failed to demonstrate a risk of PTB associated with *C. trachomatis* infections [13,14], although several large, well-conducted studies have supported a role for *C. trachomatis* in PTB [15–17]. Thus, in a large study including 4055 subjects, about 15% of PTB was attributed to *C. trachomatis* [16].

We have previously confirmed an association between *Waddlia* antibodies and human miscarriage and have demonstrated its presence in placenta and/or genital tract [4–7]. In the present study, *W. chondrophila* was not associated with PTB despite inflammation, and abnormal histology of the placenta was observed among patients infected by *Waddlia* (positive PCR). Women of African descent were more likely to have positive *Waddlia* serology. Moreover, condom use was inversely associated with *Waddlia* seropositivity.

In contrast to *Waddlia,* presence of *Chlamydia* DNA in the genital tract and/or in the placenta was strongly associated with PTB. However, *C. trachomatis* seropositivity did not correlate with PTB, suggesting that acute rather than chronic *C. trachomatis* infection is associated with PTB. Interestingly, these results are opposite to our previous findings, in which we demonstrated that both chronic and acute *C. trachomatis* infections correlate with miscarriages [18]. Our results confirm those of a population-based prospective study recently published in the Generation R cohort in the Netherlands [16]. In this large study (>4000 pregnant women), *Chlamydia* DNA was strongly associated with PTB but not with miscarriage or perinatal death. Conflicting data between *Chlamydia* DNA, serology and pregnancy outcomes may reflect different pathophysiologic mechanisms, in which *Chlamydia*-induced miscarriages are the result of an immunologic process, whereas the direct impact of the bacteria results in *Chlamydia*-induced PTB.

A limitation of our study was the absence of investigation of other infectious etiology of PTB in all patients (PTB and control subjects). Some pathogens can reach the placenta by hematogenous spread or by an ascending route from the cervix. Among bacterial infections, *Ureaplasma urealyticum, Mycoplasma hominis* and bacterial vaginosis have been associated with PTB, but controversies regarding their true role during pregnancy persist [9,19].

Overall, these results strongly suggest a role of acute *C. trachomatis* infection in PTB, and we strongly recommend systematically testing for *C. trachomatis* in any woman at risk for or after preterm delivery.

## Conflict of interest

None declared.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.nmni.2014.11.004.

## References

[1] Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012;379(9832):2162–72.

[2] Lockwood CJ. Predicting premature delivery—no easy task. N Engl J Med 2002;346:282–4.

[3] Borel N, Ruhl S, Casson N, Kaiser C, Pospischil A, Greub G. *Parachlamydia* spp. and related *Chlamydia*-like organisms and bovine abortion. Emerg Infect Dis 2007;13:1904–7.

[4] Baud D, Goy G, Osterheld MC, Borel N, Vial Y, Pospischil A, et al. *Waddlia chondrophila*: from bovine abortion to human miscarriage. Clin Infect Dis 2011;52:1469–71.

[5] Baud D, Regan L, Greub G. Emerging role of *Chlamydia* and *Chlamydia*-like organisms in adverse pregnancy outcomes. Curr Opin Infect Dis 2008;21:70–6.

[6] Baud D, Thomas V, Arafa A, Regan L, Greub G. *Waddlia chondrophila*, a potential agent of human fetal death. Emerg Infect Dis 2007;13:1239–43.

[7] Baud D, Goy G, Osterheld MC, Croxatto A, Borel N, Vial Y, et al. Role of *Waddlia chondrophila* placental infection in miscarriage. Emerg Infect Dis 2014;20:460–4.

[8] Andrews WW, Goldenberg RL, Mercer B, Iams J, Meis P, Moawad A, et al. The Preterm Prediction Study: association of second-trimester genitourinary *Chlamydia* infection with subsequent spontaneous preterm birth. Am J Obstet Gynecol 2000;183:662–8.

[9] Capoccia R, Greub G, Baud D. *Ureaplasma urealyticum, Mycoplasma hominis* and adverse pregnancy outcomes. Curr Opin Infect Dis 2013;26:231–40.

[10] Baud D, Regan L, Greub G. Comparison of five commercial serological tests for the detection of anti–*Chlamydia trachomatis* antibodies. Eur J Clin Microbiol Infect Dis 2010;29:669–75.

[11] Jaton K, Bille J, Greub G. A novel real-time PCR to detect *Chlamydia trachomatis* in first-void urine or genital swabs. J Med Microbiol 2006;55(pt 12):1667–74.

[12] Goy G, Croxatto A, Posfay-Barbe KM, Gervaix A, Greub G. Development of a real-time PCR for the specific detection of *Waddlia chondrophila* in clinical samples. Eur J Clin Microbiol Infect Dis 2009;28:1483–6.

[13] Choi SJ, Park SD, Jang IH, Uh Y, Lee A. The prevalence of vaginal microorganisms in pregnant women with preterm labor and preterm birth. Ann Lab Med 2012;32:194–200.

[14] Silveira MF, Ghanem KG, Erbelding EJ, Burke AE, Johnson HL, Singh RH, et al. *Chlamydia trachomatis* infection during pregnancy and the risk of preterm birth: a case–control study. Int J STD AIDS 2009;20:465–9.

[15] Cohen I, Veille JC, Calkins BM. Improved pregnancy outcome following successful treatment of chlamydial infection. JAMA 1990;263:3160–3.

[16] Rours GI, Duijts L, Moll HA, Arends LR, de Groot R, Jaddoe VW, et al. *Chlamydia trachomatis* infection during pregnancy associated with preterm delivery: a population-based prospective cohort study. Eur J Epidemiol 2011;26:493–502.

[17] Rours GI, de Krijger RR, Ott A, Willemse HF, de Groot R, Zimmermann LJ, et al. *Chlamydia trachomatis* and placental inflammation in early preterm delivery. Eur J Epidemiol 2011;26:421–8.

[18] Baud D, Goy G, Jaton K, Osterheld MC, Blumer S, Borel N, et al. Role of *Chlamydia trachomatis* in miscarriage. Emerg Infect Dis 2011;17:1630–5.

[19] Vouga M1, Greub G, Prod'hom G, Durussel C, Roth-Kleiner M, Vasilevsky S, et al. Treatment of genital mycoplasma in colonized pregnant women in late pregnancy is associated with a lower rate of premature labour and neonatal complications. Clin Microbiol Infect 2014;20:1074–9.