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► To cite this version:

Pierre Bonnet, Claire Hoffmann, Nathan Le Nan, Lorenn Bellamy, Gautier Hoarau, et al.. Atovaquone exposure and *Pneumocystis jirovecii* cytochrome b mutations: French data and review of the literature. *Medical Mycology*, 2023, 61 (9), 10.1093/mmy/myad095 . hal-04301211

HAL Id: hal-04301211

<https://hal.science/hal-04301211v1>

Submitted on 22 Nov 2023

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1 **Atovaquone exposure, *Pneumocystis jirovecii* cytochrome b mutations and**
2 **genotypes: French data and review of the literature**

3
4 ***Pneumocystis jirovecii* cytochrome b gene and atovaquone**

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29 **Key words:** *Pneumocystis jirovecii*, cytochrome b, *CYB*, atovaquone, selective pressure.

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50 **Summary**

51 *Pneumocystis jirovecii* is a transmissible fungus responsible for severe pneumonia
52 (*Pneumocystis pneumonia* [PCP]) in immunocompromised patients. Missense mutations due to
53 atovaquone selective pressure have been identified on cytochrome b (*CYB*) gene of *P. jirovecii*.
54 It was recently shown that atovaquone prophylaxis can lead to the selection of specific *P.*
55 *jirovecii* cytochrome b mutants potentially resistant to atovaquone among organ transplant
56 recipients. In this context, our objectives were to provide data on *P. jirovecii* cytochrome b
57 mutants and putative selective pressure exerted by atovaquone on *P. jirovecii* organisms in
58 France. A total of 123 patients (124 *P. jirovecii* specimens) from four metropolitan hospitals
59 and two overseas hospitals were retrospectively enrolled. Fourteen patients had prior exposure
60 to atovaquone whereas 109 patients did not, at the time of *P. jirovecii* detection. A 638 base-
61 pair fragment of the *CYB* gene of *P. jirovecii* was amplified and sequenced. A total of 10 SNPs
62 was identified. Both missense mutations C431T (Ala144Val) and C823T (Leu275Phe) located
63 at the Q_o active site of the enzyme, were significantly associated with prior atovaquone
64 exposure, these mutations being conversely incidental in absence of prior atovaquone exposure
65 ($P < 0.001$). Considering that the aforementioned hospitals may be representative of the national
66 territory, these findings show that overall presence of *P. jirovecii* cytochrome b mutants remains
67 low in France.

68

69 **Lay Summary**

70 The mutations C431T (Ala144Val) and C823T (Leu275Phe) located at cytochrome b active site
71 are associated with prior atovaquone exposure, these mutations being conversely incidental in
72 absence of exposure ($P < 0.001$). Overall presence of *P. jirovecii* cytochrome b mutants remains
73 low in France.

74

75 **Introduction**

76 *Pneumocystis jirovecii* is a non-cultivable, opportunistic and transmissible fungus
77 responsible for severe pneumonia (*Pneumocystis pneumonia* [PCP]) in immunocompromised
78 patients. PCP remains the most frequent acquired immunodeficiency syndrome (AIDS)
79 defining infection in patients infected with human immunodeficiency virus (HIV) in developed
80 countries. In France, at present, PCP cases in HIV-infected patients are less frequent than cases
81 in other immunosuppressed patients not infected with HIV.^{1,2} The first-line treatment and
82 prophylaxis of PCP is based on the combination of sulfamethoxazole with trimethoprim (SMX-
83 TMP), which targets the dihydropteroate synthase (DHPS) and the dihydrofolate reductase
84 (DHFR) respectively, two enzymes involved in folate biosynthesis. Two major missense
85 mutations that occur at nucleotide positions 165 and 171, have been described at the *dhps* locus
86 of *P. jirovecii*.³ Variants harboring these mutations have been associated with sulfonamide
87 exposure and putative resistance of *P. jirovecii* organisms to these drugs.⁴ Currently, prior
88 exposure to sulfonamides and the city of patient residence have been identified as the two main
89 predictors of *P. jirovecii* DHPS mutants worldwide.^{5,6} Moreover, occasional severe adverse
90 effects of SMX-TMP lead to the use of second-line treatments or prophylaxis. Atovaquone is
91 an alternative, used for both PCP treatment and prophylaxis in the USA, mainly in patients with
92 hematological malignancies or solid organ transplants. It is only an approved second-line drug
93 for PCP treatment in France but despite this restriction it is also used as PCP prophylaxis in
94 patients that cannot be given first-line therapy. This drug is a competitive inhibitor of b subunit
95 of the cytochrome bc₁ enzyme complex. This complex located into the mitochondrial
96 membrane participates in the electron transfers necessary for the functioning of the respiratory
97 chain.⁷ Missense mutations in cytochrome b gene Ile147Val, Thr148Ile, Leu150Phe,
98 Ser152Ala, Pro266Leu, and Leu275Phe have been observed in *P. jirovecii* strains, and other
99 mutations were found originally in *Saccharomyces cerevisiae* (Leu275Phe/Ser/Thr),

100 *Plasmodium yoelii* (Tyr279Cys), and *P. falciparum* (Tyr279Ser).⁸⁻¹⁰ The aforementioned
101 mutations in *P. jirovecii* and *Plasmodium* sp. confer reduced sensitivity to atovaquone in
102 enzymatic models from *S. cerevisiae*.⁸⁻¹⁰ Moreover, failure of prophylaxis based on the use of
103 atovaquone in patients at risk for PCP has been documented.¹¹⁻¹⁵ Specifically, Argy and
104 colleagues recently described a previously unreported A144V mutation (C431T) that
105 diminished the sensitivity of *P. jirovecii* to atovaquone, that may explain the spread of the
106 fungus among heart transplant recipients receiving atovaquone prophylaxis.¹⁴
107 The objectives of the present study were to provide data on *P. jirovecii* cytochrome b mutants
108 and information on putative selective pressure exerted by atovaquone on *P. jirovecii* organisms
109 in France. In this context, we retrospectively analyzed the *CYB* gene of a collection of *P.*
110 *jirovecii* specimens in patients from six French hospitals, exposed or not exposed to
111 atovaquone, at the time of *P. jirovecii* detection. We also provided a review of the literature on
112 *P. jirovecii* *CYB* diversity and the description of new alleles.

113 **Materials and Methods**

114 A total of 123 patients (124 *P. jirovecii* specimens) who tested positive for *P. jirovecii*
115 infection were retrospectively enrolled in the study (Table 1). They were monitored from
116 February 2009 to March 2022. Seventy-eight *P. jirovecii* specimens were from patients
117 monitored in Brest University Hospital. The remaining 46 *P. jirovecii* specimens were from
118 patients monitored in five other hospitals, three metropolitan hospitals, Nantes (six patients),
119 Paris Bichat hospital (19 patients), Saint-Étienne (one patient) and two overseas hospitals,
120 Cayenne, French Guiana (six patients), and Réunion island (14 patients). *P. jirovecii* was
121 initially detected by microscopic examination of pulmonary specimens and/or PCR assays.
122 Ninety-four patients were considered to develop overt PCP, eight patients were considered to
123 be colonized by the fungus, and 21 infants were considered to develop a primary infection. *P.*

124 *jirovecii* in colonized patients was not detected by microscopy while it was detected by PCR.

125 The colonized patients presented with alternative diagnoses of PCP.

126 Fourteen patients had prior atovaquone exposure (first group). Atovaquone exposure
127 was defined on the basis of the patients' prior exposure during the year before *P. jirovecii*
128 detection, whatever the atovaquone dosage and its duration. The median age in this group was
129 53 years (range, 35 to 81 years); the male-female ratio was 9/5. Underlying conditions were
130 solid-organ transplantation (eight patients), hematological malignancy (two patients), solid-
131 organ transplantation and HIV (one patient), giant cell arteritis (one patient), epidermoid
132 carcinoma (one patient) and Myasthenia gravis (one patient) (Tables 1 and 2). The second group
133 comprised 109 patients who had no known prior exposure to atovaquone at the time of *P.*
134 *jirovecii* detection (second group). This group included patients without history of atovaquone
135 treatment or prophylaxis within the year before *P. jirovecii* detection. The median age was 61
136 years (range, 0.1 to 89 years); the male-female ratio was 74/35. Underlying conditions included
137 organ transplantation (26 patients), HIV infection (22 patients), hematological malignancy (14
138 patients), cancer (11 patients), autoimmune disease (seven patients), premature birth (four
139 patients), interstitial lung disease (three patients), lymphopenia (two patients), congenital heart
140 disease (two patients), hypersensitivity pneumonitis (one patient), non-X histiocytosis (one
141 patient), West syndrome (one patient), tetralogy of Fallot (one patient), primary
142 immunodeficiency (one patient), laryngomalacy (one patient), Down's syndrome (one patient),
143 esophageal carcinoma and COPD (Chronic Obstructive Pulmonary Disease) (one patient), and
144 unknown underlying condition (10 patients) (Table 1 and Supplementary Table S1). Some
145 patients may have been previously enrolled in other studies and their data described in part
146 elsewhere.^{13-15,18-21} *P. jirovecii* DNA specimens from the patients were stored at -20 ° C until
147 further examination.

148 The primer pair CytbFw and CytbRw was used to amplify a section of the *CYB* gene as
149 described elsewhere.¹⁶ PCR reactions were carried out as previously described.¹³ The expected
150 size of amplicons was 638 bp. To avoid contamination, each step was performed in different
151 areas with different sets of micropipettes. The reagents used in the PCR mixtures were prepared
152 in a laminar-flow cabinet. To monitor for possible contamination, negative controls were
153 included. PCR products were purified using ExoSAP-IT® (Affymetrix Inc., US) and sequenced
154 from the two strands using the dideoxy chain termination method (BigDye Terminator version
155 1.1 cycle sequencing kit, Applied Biosystems, Foster City, California) on the ABI Prism 3130xl
156 DNA sequencer (Applied Biosystems, Courtaboeuf, France). DNA sequences of *CYB* were
157 edited using Pregap and Gap software (Staden package version 2.0.0b11-2016; Staden
158 Group).²² Consensus sequences were aligned with the reference sequence (GenBank accession
159 number AF074871)¹¹ using the BioEdit software with the Clustal W program. The putative
160 nucleotide positions were not those initially numbered by Walker and colleagues to describe
161 the SNPs.¹¹ We renumbered them considering the whole *CYB* gene included in the *P. jirovecii*
162 mitochondrial genome assembly from Ma and colleagues (GenBank accession number
163 JX855938) (Table 3).²³ Alleles were named using the nomenclature based on scoring nucleotide
164 positions 360, 380, 429, 443, 450, 597, 628, 647, 756, 823, 913, 914, and 919, as described
165 elsewhere.¹⁵⁻¹⁷

166 Statistical analyses were performed using Prism software (version 7.0; GraphPad
167 Software, San Diego, CA, USA). Qualitative variables were compared using Fisher's exact test.
168 A two-sided P value of 0.05 was considered significant. Ethical review and approval were
169 waived for this study, in accordance with the French public health law (CSP Art L1121e1.1),
170 because this study was non-interventional, focused on *P. jirovecii* specimens, and used
171 samplings and data obtained from the patients' standard routine care.

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173 **Results**

174 *P. jirovecii* *CYB* nucleotide sequences of the 124 patients (125 specimens) were
175 obtained and compared with the wild-type reference sequence (GenBank accession number
176 AF074871).¹¹ Overall, 10 SNPs were observed at nucleotide positions 360 (C or T), 363 (C or
177 T), 429 (A or G), 431 (C or T), 597 (C or T), 628 (C or T), 647 (C or T), 823 (C or T), 846 (G
178 or A), and 919 (C or T) (Table 2 and Supplementary Table S1). SNPs C360T, A429G, C597T,
179 and C628T, correspond to previously described synonymous mutations that were not involved
180 in amino acid changes (Tables 2, 3, and Supplementary Tables S1, S2, S3).^{11,12,24} They were
181 observed in 17, four, one, and 26 patients of the second patient group, respectively. Two
182 previously undescribed SNPs, C363T and G846A, corresponding to synonymous mutations
183 were found in one and two patients of the second patient group, respectively (GenBank
184 accession numbers OR192130 and OR192131, respectively). The SNP at position 363 was
185 detected in only one patient. It was confirmed with a second PCR-sequencing experiment.
186 Moreover, one SNP at position 597 was also detected in one patient of the first group.

187 Four SNPs that corresponded to four previously described missense mutations [C431T
188 (Ala144Val), C647T (Ser216Leu), C823T (Leu275Phe), and C919T (Leu307Phe)] were
189 identified (Tables 2, 3, and Supplementary Tables S2 and S3). Both C431T (Ala144Val) and
190 C823T (Leu275Phe) located at Q_o site of the mitochondrial cytochrome bc₁, the active site of
191 the enzyme, were identified in eight and three patients of the first patient group respectively
192 (one patient harbored both mutations). C431T (Ala144Val) was also evidenced in two patients
193 from the second group. C647T (Ser216Leu) was only found in second patient group (four
194 patients). C919T (Leu307Phe) was found in both patient groups (in eight and 43 patients,
195 respectively). These latter mutations were not located at Q_o site of the enzyme. Mixed infections
196 were observed in 23 patients, one of the first group and 22 of the second group. Mixed infections

197 are defined by the presence of more than one SNP at more than one nucleotide position
198 simultaneously in the same *P. jirovecii* specimen.

199 Overall, 10 *CYB* alleles named CYB1 to CYB8, CYB10, and CYB11 were identified
200 (Table 2 and Supplementary Tables S1, S2, S3). CYB1 and CYB2 were the most frequent
201 alleles in the second group of patients as they were observed in 46 (36.5%) and 37 (29.4%)
202 patients, respectively. Seven *P. jirovecii* strains from the first group harbored CYB2 allele with
203 an additional C431T (Ala144Val) missense mutation. This allele CYB2 could be renamed as a
204 new allele CYB12, which was the most frequent allele in the first group. Alleles corresponding
205 to the previously undescribed SNPs C363T and G846A could be renamed as new alleles CYB13
206 and CYB14, respectively. The allele was not determined in patients B4, B26, B27, B37, B56,
207 S1, and LR12 because of putative mixed infections revealed by more than one SNPs at more
208 than one nucleotide position simultaneously. A significant difference was observed between the
209 two patient groups, atovaquone exposed vs. atovaquone non-exposed at the time of *P. jirovecii*
210 detection, when comparing the repartition of alleles including synonymous mutations (CYB1,
211 CYB3 to CYB6, CYB8, CYB13, and CYB14), alleles including missense mutations far from
212 the active site (CYB2, CYB7, and CYB10) and alleles including missense mutations at the Q_o
213 site of the enzyme (CYB11 and CYB12) (Fisher's exact test, $P < 0.001$). These findings show
214 that atovaquone exposure leads to allele selection, specifically those harboring missense
215 mutations at the Q_o site. Nonetheless, if we consider only the patients effectively cured with
216 atovaquone for PCP treatment, none of the aforementioned allele groups was significantly
217 associated with improvement or deterioration (Fisher's exact test, $P = 0.19$).

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221 Discussion

222 Mutations in *P. jirovecii* strains potentially resulting from atovaquone prior exposure
223 have been already reported.¹¹⁻¹⁵ Here we assessed the selective pressure exerted by atovaquone
224 on *P. jirovecii* through a French multicenter study. We chose an approach that compared
225 specifically patients with prior atovaquone exposure and patients without prior atovaquone
226 exposure, at the time of PCP, *P. jirovecii* colonization or primary infection diagnoses. The
227 association of the two previously described missense mutations C431T (Ala144Val) and C823T
228 (Leu275Phe) located at Q_o active site of the mitochondrial cytochrome bc₁,¹²⁻¹⁵ with prior
229 atovaquone exposure was strengthened. Indeed, none of patients without atovaquone exposure
230 were infected with *P. jirovecii* harboring the aforementioned mutations, except the two patients
231 who contracted the fungus in a context of nosocomial acquisition during an outbreak.¹⁴

232 If the following missense mutations Gly143Ala, Ile147Val, Thr148Ile, Leu150Phe,
233 Ser152Ala, Pro266Leu, Leu275Phe/Ser, and Tyr279Cys/Ser in the CYB gene that confer *in*
234 *vitro* resistance to atovaquone have already been described,⁸⁻¹⁰ to the best of our knowledge no
235 study highlighting clinical failure of PCP atovaquone treatment related to *P. jirovecii* CYB
236 mutant strains is presently available. Our study did not bring any additional data of this topic.

237 Two previously undescribed SNPs were found in this study in patients without prior
238 atovaquone exposure at nucleotide positions 363 (C or T) and 846 (G or A), both located far
239 from the active site of the cytochrome b subunit. They may reflect genomic diversity and are
240 less likely to be involved in atovaquone selective pressure. Nonetheless, it has been suggested
241 elsewhere that synonymous mutations may affect protein translational kinetics and drug-target
242 interactions.²⁸⁻³⁰ We did not consider this field of investigation in this study.

243 A significant difference in allele distribution between the two patient groups, atovaquone
244 exposed vs. atovaquone non-exposed at the time of *P. jirovecii* detection, was observed. There

245 was a different distribution between alleles related to synonymous mutations, alleles related to
246 missense mutations out of the active site and alleles related to missense mutations at the active
247 site of the enzyme (Fisher's exact test, $P < 0.001$). These results suggest that atovaquone
248 exposure exerts a selective pressure on *P. jirovecii* organisms revealed by missense mutations
249 at Q_o site of its target gene. Nonetheless, overstatement of the presence of C431T (Ala144Val)
250 from CYB12 in the first patient group, and consequently of this putative selective pressure of
251 atovaquone is possible since nine of patients monitored in Paris Bichat hospital were involved
252 in an outbreak in the course of which a *P. jirovecii* strain harboring this mutation was
253 transmitted.

254 The low number of patients enrolled in this retrospective study may represent its limit.
255 Indeed, despite the enrollment of patients from six French centers, only 14 patients met the
256 criteria for being enrolled in the first patient group, i.e. atovaquone-exposed patients. This is
257 explained by the fact that atovaquone remains a second-line therapy or prophylaxis for PCP.
258 Despite $P < 0.001$ as shown by Fisher's exact test, our analysis may lack power. Moreover, the
259 low frequency of cytochrome b mutants observed in the patient population not exposed to
260 atovaquone could be due to the lack of sensitivity of Sanger sequencing. Indeed, next-
261 generation sequencing was found to be more effective in detecting minor haplotypes.
262 Nonetheless, we detected mixed infections in 45/123 patients using Sanger sequencing which
263 appeared efficient enough in detecting potential minor haplotypes.

264 One patient from the first group was infected with *P. jirovecii* organisms harboring the
265 SNP C431T (Ala144Val) but was not concerned by the cluster that occurred in Paris Bichat
266 hospital. The patient was monitored in another French hospital, Saint-Étienne University
267 Hospital, 550 km from Paris. *P. jirovecii* organisms in this patient harbored also the SNP C823T
268 (Leu275Phe). The two patients infected with *P. jirovecii* organisms that harbored C823T
269 (Leu275Phe) were monitored in Réunion University Hospital, 9400 km from metropolitan

270 France, were. These three patients belong to the first patient group and had prior atovaquone
271 exposure at the time of the *P. jirovecii* detection. It is noteworthy that the nucleotide change
272 C823T was previously observed by Kazanjian and colleagues in a *P. jirovecii* specimen from a
273 patient who presented with prior atovaquone exposure.¹² Be that as it may, these observations
274 are in favor of the implication of atovaquone exposure in the selection of mutants harboring
275 C431T (Ala144Val) and C823T (Leu275Phe) mutations, located at the active site of
276 cytochrome b subunit.

277 These results brought additional data to that of Walker et al.¹¹ and Kazanjian et al.,¹²
278 from years 1998 and 2001, respectively. Both studies described the first SNPs in the *CYB*
279 potentially related to atovaquone prophylaxis failure, C380T (Thr127Ile), A439G (Ile147Val),
280 C443T (Thr148Ile), G450T (Leu150Phe), T454G (Ser152Ala), C797T (Pro266Leu), and
281 C823T (Leu275Phe). They did not name corresponding *CYB* alleles. The present study
282 describes again atovaquone PCP prophylaxis failure in patients with prior atovaquone exposure,
283 potentially due to missense mutations C823T (Leu275Phe) and C431T (Ala144Val) located at
284 the active site of the cytochrome b enzyme, the target of atovaquone. Esteves et al.,¹⁶ Maitte et
285 al.,¹⁷ and Le Gal et al.¹⁵ named for the first time *CYB* alleles from CYB1 to CYB11 in years
286 2010, 2014, and 2020, respectively. In the present study, we propose to name new genotypes,
287 from CYB12 to CYB14.

288 In conclusion, the results show that direct selective pressure exerted by atovaquone
289 among *P. jirovecii* microorganisms in patients with prior exposure to the drug is high.
290 Conversely, the indirect selective pressure of atovaquone which could have been revealed by
291 the circulation and the presence of cytochrome b *P. jirovecii* mutants in patients without prior
292 exposure to the drug remains incidental and does not represent a public health issue in France
293 so far.

294 **Acknowledgments**

295 The authors would like to thank Mr André Happe, Mr Stéphane Gouriou, and the Brest
296 University Hospital Health Data Warehouse for their contribution to the data collection.

297

298 **Conflicts of Interest**

299 The authors report no conflicts of interest. The authors alone are responsible for the content and
300 the writing of the paper.

301

302 **References**

303

304 1. Totet A, Damiani C, Le Gal S, Nevez G. Infections à *Pneumocystis jirovecii* : biologie
305 et clinique. *Encyclopédie médico-chirurgicale - Maladies infectieuses*. 2021; Article 8-
306 590-A-10.

307

308 2. Cazein F, Pillonel J, Sommen C et al. Découvertes de séropositivité VIH et diagnostics
309 de sida - France, 2018. *Bulletin de santé publique*. 2019;
310 [https://www.santepubliquefrance.fr/maladies-et-traumatismes/infections-](https://www.santepubliquefrance.fr/maladies-et-traumatismes/infections-sexuellement-transmissibles/vih-sida/documents/bulletin-national/bulletin-de-sante-publique-vih-sida-octobre-2019)
311 [sexuellement-transmissibles/vih-sida/documents/bulletin-national/bulletin-de-sante-](https://www.santepubliquefrance.fr/maladies-et-traumatismes/infections-sexuellement-transmissibles/vih-sida/documents/bulletin-national/bulletin-de-sante-publique-vih-sida-octobre-2019)
312 [publique-vih-sida-octobre-2019](https://www.santepubliquefrance.fr/maladies-et-traumatismes/infections-sexuellement-transmissibles/vih-sida/documents/bulletin-national/bulletin-de-sante-publique-vih-sida-octobre-2019).

313

314 3. Lane BR, Ast JC, Hossler PA et al. Dihydropteroate synthase polymorphisms in
315 *Pneumocystis carinii*. *J Infect Dis*. 1997; 175: 482-485.

316

- 317 4. de la Horra C, Friaiza V, Morilla R et al. Update on Dihydropteroate Synthase (DHPS)
318 Mutations in *Pneumocystis jirovecii*. *J Fungi (Basel)*. 2021; 7: 856.
319
- 320 5. Beard CB, Carter JL, Keely SP et al. Genetic variation in *Pneumocystis carinii* isolates
321 from different geographic regions: implications for transmission. *Emerg Infect Dis*.
322 2000; 6: 265-272.
323
- 324 6. Huang L, Beard CB, Creasman J et al. Sulfa or sulfone prophylaxis and geographic
325 region predict mutations in the *Pneumocystis carinii* dihydropteroate synthase gene. *J*
326 *Infect Dis*. 2000; 182: 1192-1198.
327
- 328 7. Musso L, Fabbrini A, Dallavalle S. Natural Compound-derived Cytochrome bc1
329 Complex Inhibitors as Antifungal Agents. *Molecules*. 2020; 25: 4582.
330
- 331 8. Hill P, Kessl J, Fisher N. Recapitulation in *Saccharomyces cerevisiae* of cytochrome b
332 mutations conferring resistance to atovaquone in *Pneumocystis jirovecii*. *Antimicrob*
333 *Agents Chemother*. 2003; 47: 2725–2731.
334
- 335 9. Kessl JJ, Hill P, Lange BB. Molecular basis for atovaquone resistance in *Pneumocystis*
336 *jirovecii* modeled in the cytochrome bc(1) complex of *Saccharomyces cerevisiae*. *J Biol*
337 *Chem*. 2004; 279: 2817-2824.
338
- 339 10. Fisher N, Meunier B. Molecular basis of resistance to cytochrome bc1 inhibitors. *FEMS*
340 *Yeast Res*. 2008; 8: 183-192.
341

- 342 11. Walker DJ, Wakefield AE, Dohn MN et al. Sequence polymorphisms in the
343 *Pneumocystis carinii* cytochrome b gene and their association with atovaquone
344 prophylaxis failure. *J Infect Dis.* 1998; 178: 1767-1775.
- 345
- 346 12. Kazanjian P, Armstrong W, Hossler PA et al. *Pneumocystis carinii* cytochrome b
347 mutations are associated with atovaquone exposure in patients with AIDS. *J Infect Dis.*
348 2001; 183: 819-822. Erratum in: *J Infect Dis.* 2001; 183: 1170.
- 349
- 350 13. Vindrios W, Argy N, Le Gal S et al. Outbreak of *Pneumocystis jirovecii* Infection
351 Among Heart Transplant Recipients: Molecular Investigation and Management of an
352 Interhuman Transmission. *Clin Infect Dis.* 2017; 65: 1120-1126.
- 353
- 354 14. Argy N, Le Gal S, Coppée R et al. *Pneumocystis* cytochrome b mutants associated with
355 atovaquone prophylaxis failure as the cause of *Pneumocystis* infection outbreak among
356 heart transplant recipients. *Clin Infect Dis.* 2018; 67: 913-919.
- 357
- 358 15. Le Gal S, Hoarau G, Bertolotti A et al. *Pneumocystis jirovecii* Diversity in Réunion, an
359 Overseas French Island in Indian Ocean. *Front Microbiol.* 2020; 11: 127.
- 360
- 361 16. Esteves F, Gaspar J, Tavares A et al. Population structure of *Pneumocystis jirovecii*
362 isolated from immunodeficiency virus-positive patients. *Infect Genet Evol.* 2010; 10:
363 192-199.
- 364
- 365 17. Maitte C, Leterrier M, Le Pape P, Miegerville M, Morio F. Multilocus sequence typing
366 of *Pneumocystis jirovecii* from clinical samples: how many and which loci should be
367 used? *J Clin Microbiol.* 2013; 51: 2843–2849.

368
369
370
371
372
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374
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380
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382
383
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391
392

18. Le Gal S, Blanchet D, Damiani C et al. AIDS-related *Pneumocystis jirovecii* genotypes in French Guiana. *Infect Genet Evol.* 2015; 29: 60-67.

19. Nevez G, Guillaud-Saumur T, Cros P et al. *Pneumocystis* primary infection in infancy: Additional French data and review of the literature. *Med Mycol.* 2020; 58: 163-171.

20. Hoffmann CV, Nevez G, Moal MC et al. Selection of *Pneumocystis jirovecii* Inosine 5'-Monophosphate Dehydrogenase Mutants in Solid Organ Transplant Recipients: Implication of Mycophenolic Acid. *J Fungi (Basel).* 2021; 7: 849.

21. Nevez G, Morio F, Bonnet P et al. A proposal for pragmatic investigation of possible clonal clusters of *Pneumocystis* pneumonia cases. *J Hosp Infect.* 2021; 108: 215-216.

22. Bonfield JK, Staden R. Experiment files and their application during large-scale sequencing projects. *DNA Seq.* 1996; 6: 109–117.

23. Ma L, Huang DW, Cuomo CA et al. Sequencing and characterization of the complete mitochondrial genomes of three *Pneumocystis* species provide new insights into divergence between human and rodent *Pneumocystis*. *FASEB J.* 2013; 27: 1962-1972.

24. Takahashi T, Kanda T, Iwamoto A. Genetic diversity of drug targets including dihydropteroate synthase, dihydrofolate reductase and cytochrome b, in *Pneumocystis carinii* f. sp. hominis isolates in Japan. *Res Commun Mol Pathol Pharmacol.* 2002; 112: 159-176.

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410
411
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415
416

25. Charpentier E, Garnaud C, Wintenberger C et al. Added Value of Next-Generation Sequencing for Multilocus Sequence Typing Analysis of a *Pneumocystis jirovecii* Pneumonia Outbreak¹. *Emerg Infect Dis.* 2017; 23: 1237-1245.
26. Cissé OH, Ma L, Wei Huang D et al. Comparative Population Genomics Analysis of the Mammalian Fungal Pathogen *Pneumocystis*. *mBio.* 2018; 9: e00381-18.
27. Monroy-Vaca EX, de Armas Y, Illnait-Zaragozí MT et al. Genetic diversity of *Pneumocystis jirovecii* in colonized Cuban infants and toddlers. *Infect Genet Evol.* 2014; 22: 60-66.
28. Kimchi-Sarfaty C, Oh JM, Kim IW et al. A “silent” polymorphism in the MDR1 gene changes substrate specificity. *Science.* 2017; 315: 525–528.
29. Harrigan PR, Sheen CW, Gill VS et al. Silent mutations are selected in HIV-1 reverse transcriptase and affect enzymatic efficiency. *AIDS.* 2008; 22: 2501–2508.
30. Callens M, Pradier L, Finnegan M, Rose C, Bedhomme S. Read between the lines: diversity of nontranslational selection pressures on local codon usage. *Genome Biol Evol.* 2021; 13: evab097.

Table 1. Characteristics of 123 patients with or without prior atovaquone exposure at the time of *Pneumocystis jirovecii* infection diagnoses

	Patients with prior atovaquone exposure at the time of <i>P. jirovecii</i> infection diagnoses	Patients without prior atovaquone exposure at the time of <i>P. jirovecii</i> infection diagnoses
No. of patients	14	109
Median age [range]	53 [35-81]	61 [0.1-89]
Sex ratio (M/F)	9/5	74/35
Samples	BAL (n = 9) ; Sputum (n = 3) ; NS (n = 2)	BAL (n = 77) ; Sputum (n = 10) ; NS (n = 1) ; ETA (n = 1) ; NPA (n = 21)
Presentation of <i>Pneumocystis</i> infection	PCP (n = 12) ; colonization ¹ (n = 2)	PCP (n = 82) ; colonization ¹ (n = 6) ; primary infection (n = 21)
Underlying conditions (No. of patients)	SOT (n = 8) ; SOT and HIV (n = 1) ; H (n = 2) ; AI (n = 2) ; Cancer (n = 1)	SOT (n = 26) ; HIV (n = 22) ; H (n = 14) ; Cancer (n = 11) ; AI (n = 7) ; Premature birth (n = 4) ; Cancer and COPD (n = 1) ; Others ² (n = 14) ; None ³ (n = 10)

BAL, bronchoalveolar lavage; NS, nasopharyngeal swab; ETA, endotracheal aspiration; NPA, nasopharyngeal aspiration; PCP, *Pneumocystis* pneumonia; M, male; F, female; SOT, solid organ transplant; H, hemopathy; AI, autoimmune disease; HIV, human immunodeficiency virus; COPD, Chronic Obstructive Pulmonary Disease

¹ *P. jirovecii* was not detected by microscopic examination, it was only detected by PCR assay. The patients presented alternative diagnoses of PCP.

² Interstitial lung disease (n = 3) ; Lymphopenia (n = 2) ; Congenital heart disease (n = 2) ; Hypersensitivity pneumonitis (n = 1) ; Non-X histiocytosis (n = 1) ; West syndrome (n = 1) ; Tetralogy of Fallot (n = 1) ; Primary immunodeficiency (n = 1) ; Laryngomalacia (n = 1) ; Down's syndrome (n = 1)

³ Absence of immunosuppression, neonates who developed *Pneumocystis* primary infection.

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Table 2. Characteristics of 14 patients with prior exposure to atovaquone at the time of *Pneumocystis jirovecii* infection diagnoses and *P. jirovecii* alleles at the cytochrome b gene

Patient code ^a	Underlying condition(s)	Patient still under atovaquone at the time of <i>Pneumocystis</i> infection diagnoses	Sample	Presentation of <i>Pneumocystis</i> infection	Treatment of <i>Pneumocystis</i> infection	Outcome	CYB allele ^j	Missense mutation(s) at the Qo active site
P1	Kidney and heart transplant, HIV ^b	Yes	BAL ^c	PCP ^e	SMX-TMP ^g	Improvement	CYB12 ^k	C431T ^m
P2	Heart transplant	Yes	Sputum	PCP	SMX-TMP	Improvement	CYB12	C431T
P3	Heart transplant	Yes	BAL	PCP	SMX-TMP	Improvement	CYB12	C431T
P4	Heart transplant	Yes	BAL	PCP	SMX-TMP, atovaquone and pentamidine	Improvement	CYB12	C431T
P5	Heart transplant	Yes	BAL	PCP	SMX-TMP	Improvement	CYB12	C431T
P6	Heart transplant	Yes	NS ^d	PCP	SMX-TMP	Improvement	CYB12	C431T
P7	Heart transplant	Yes	NS	Colonization ^f	- ^h	Improvement	CYB12	C431T
P8	Heart transplant	Yes	BAL	Colonization	- ^h	Deterioration	CYB1	-
P9	Epidermoid carcinoma	Yes	Sputum	PCP	SMX-TMP	Deterioration	CYB6	-
LR1	Myeloma	Yes	BAL	PCP	Atovaquone, pentamidine	NA ⁱ	CYB11	C823T ^m
LR2	Acute lymphoblastic leukemia	Yes	BAL	PCP	SMX-TMP	NA	CYB11	C823T
B1	Giant cell arteritis	Yes	Sputum	PCP	SMX-TMP, Atovaquone	Improvement	CYB1	-
B2	Kidney transplant	Yes	BAL	PCP	Pentamidine	Deterioration	CYB2	-
S1	Myasthenia gravis	Yes	BAL	PCP	Atovaquone, SMX-TMP	Improvement	Mixed ^l	C431T and C823T

^a Patients P1 to P9 were monitored at Bichat–Claude Bernard hospital, Paris, France, and correspond to the same cluster during a *P. jirovecii* infection outbreak. Patients LR1 and LR2 were monitored at South Réunion Island University Hospital, Réunion Island, France. Patients B1 and B2 were monitored at Brest University Hospital, Brest, France. Patient S1 was monitored at Saint-Étienne University Hospital, Saint-Étienne, France. were examined at the cytochrome b gene.

^b HIV, Human immunodeficiency virus.

^c BAL, Bronchoalveolar lavage.

^d NS, Nasopharyngeal swab.

^e PCP, *Pneumocystis* pneumonia.

^f *P. jirovecii* was not detected by microscopic examination, it was only detected by PCR assay. The patients presented alternative diagnoses of PCP.

^g SMX-TMP, Sulfamethoxazole-trimethoprim.

^h Patients P7 and P8 did not receive any specific treatment against *P. jirovecii* infection since they were considered to be colonized.

ⁱ NA, Not available.

^j CYB, cytochrome b. Alleles were named using scoring nucleotide positions 597, 823, and 919, as previously described.¹⁵⁻¹⁷

^k Initially named CYB2 considering nucleotide position 919,¹⁶ and renamed CYB12 in this study considering additional SNP at nucleotide position 431.

^l Allele was not determined because of putative mixed infections revealed by more than one SNP at more than one nucleotide position simultaneously.

^m SNPs C431T and C823T from the CYB whole gene corresponded respectively to nucleotide positions C350T and C742T using the partial reference sequence (GenBank accession number AF074871).¹¹

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Table 3. Scoring positions and corresponding *CYB* alleles found in this study in patients with or without prior atovaquone exposure at the time of *Pneumocystis jirovecii* infection diagnoses

Nucleotide positions																Prior atovaquone exposure at the time of <i>P. jirovecii</i> infection (%)	No prior atovaquone exposure at the time of <i>P. jirovecii</i> infection (%)	Total (%)	
360 ^a	363	380	429	431	443	450	597	628	647	756	823	846	913	914	919	Allele			
C	C	C	A	C	C	G	C	C	C	A	C	G	T	T	C	CYB1	2 (14.3)	46 (36.5)	48 (34.3)
C	C	C	A	C	C	G	C	C	C	A	C	G	T	T	T	CYB2	1 (7.1)	37 (29.4)	38 (27.1)
C	C	C	G	C	C	G	T	C	C	A	C	G	T	T	C	CYB3	0	2 (1.6)	2 (1.4)
C	C	C	A	C	C	G	C	T	C	A	C	G	T	T	C	CYB4	0	1 (0.8)	1 (0.7)
T	C	C	A	C	C	G	T	C	C	A	C	G	T	T	C	CYB5	0	5 (4)	5 (3.6)
C	C	C	A	C	C	G	T	C	C	A	C	G	T	T	C	CYB6	1 (7.1)	11 (8.7)	12 (8.6)
C	C	C	A	C	C	G	C	C	T	A	C	G	T	T	C	CYB7	0	4 (3.2)	4 (2.9)
T	C	C	A	C	C	G	C	C	C	A	C	G	T	T	C	CYB8	0	8 (6.3)	8 (5.7)
C	C	C	A	C	C	G	C	C	C	T	C	G	T	T	C	CYB10	0	2 (1.6)	2 (1.4)
C	C	C	A	C	C	G	C	C	C	A	T	G	T	T	C	CYB11	2 (14.3)	0	2 (1.4)
C	C	C	A	T	C	G	C	C	C	A	C	G	T	T	T	CYB12	7 (50)	2 (1.6)	9 (6.4)
C	T	C	A	C	C	G	C	C	C	A	C	G	T	T	C	CYB13	0	1 (0.8)	1 (0.7)
C	C	C	A	C	C	G	C	C	C	A	C	A	T	T	C	CYB14	0	1 (0.8)	1 (0.7)
Undetermined ^b																1 (7.1)	6 (4.8)	7 (5)	

^a Alleles were named using scoring nucleotide positions 360, 380, 429, 443, 450, 597, 628, 647, 756, 823, 913, 914, and 919, as previously described.¹⁵⁻¹⁷ Nucleotide positions 363 and 846 were used for the first time in this study for the description of alleles CYB13 and CYB14, respectively.

^b Allele was not determined because of putative mixed infections revealed by more than one SNP at more than one nucleotide position simultaneously.