

Lessons from new mouse models of glycogen storage disease type 1a in relation to the time course and organ specificity of the disease

Fabienne Rajas, Julie Clar, Amandine Gautier-Stein, Gilles Mithieux

► **To cite this version:**

Fabienne Rajas, Julie Clar, Amandine Gautier-Stein, Gilles Mithieux. Lessons from new mouse models of glycogen storage disease type 1a in relation to the time course and organ specificity of the disease. *Journal of Inherited Metabolic Disease*, Springer Verlag, 2015, 38 (3), pp.521-527. <10.1007/s10545-014-9761-0>. <inserm-01350960>

HAL Id: inserm-01350960

<http://www.hal.inserm.fr/inserm-01350960>

Submitted on 2 Aug 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Lessons from new mouse models of glycogen storage disease type 1a
in relation to the time course and organ specificity of the disease

Fabienne Rajas¹⁻³, Julie Clar¹⁻³, Amandine Gautier-Stein¹⁻³ and Gilles Mithieux¹⁻³

¹Institut National de la Santé et de la Recherche Médicale, U855, Lyon, F-69008, France

²Université de Lyon, Lyon, F-69008 France

³Université Lyon1, Villeurbanne, F-69622 France

Address for correspondence: Dr. Fabienne Rajas
Inserm U855/University Lyon 1 Laennec
7 rue Guillaume Paradin
69372 Lyon cedex 08 France
Tel: 33 478 77 10 28/Fax: 33 478 77 87 62
E-mail: fabienne.rajas@univ-lyon1.fr

Word count: 2985

Number of figures: 1 Figure and 1 Table

Summary

1
2 Patients with glycogen storage diseases type 1 (GSD1) suffer from life-threatening
3
4 hypoglycaemia, when left untreated. Despite an intensive dietary treatment, patients develop
5
6 severe complications, such as liver tumors and renal failure, with aging. Until now, the animal
7
8 models available for studying the GSD1 did not survive after weaning. To gain further
9
10 insights into the molecular mechanisms of the disease and to evaluate potential treatment
11
12 strategies, we have recently developed novel mouse models in which the catalytic subunit of
13
14 glucose-6 phosphatase (*G6pc*) is deleted in each glucose-producing organ specifically. For
15
16 that, B6.*G6pc*^{ex3lox/ex3lox} mice were crossed with transgenic mice expressing a recombinase
17
18 under the control of the serum albumin, the kidney androgen protein or the villin promoter, in
19
20 order to obtain liver, kidney or intestine *G6pc*^{-/-} mice, respectively.
21
22
23
24
25

26 As opposed to total *G6pc* knockout mice, tissue-specific *G6pc* deficiency allows mice
27
28 to maintain their blood glucose by inducing glucose production in the other gluconeogenic
29
30 organs. Even though it is considered that glucose is produced mainly by the liver, liver *G6pc*^{-/-}
31
32 mice are perfectly viable and exhibit the same hepatic pathological features as GSD1 patients,
33
34 including the late development of hepatocellular adenomas and carcinomas. Interestingly,
35
36 renal *G6pc*^{-/-} mice developed renal symptoms similar to the early human GSD1 nephropathy.
37
38 This includes glycogen overload that leads to nephromegaly and morphological and
39
40 functional alterations in the kidneys. Thus, our data suggest that renal G6Pase deficiency *per*
41
42 *se* is sufficient to induce the renal pathology of GSD1.
43
44
45
46
47

48 Therefore, these new mouse models should allow us to improve the strategies of
49
50 treatment on both nutritional and pharmacological points of view.
51
52
53

54
55 **Synopsis:** Mice with tissue-specific G6Pase deletion allowed the reproduction of the hepatic
56
57 and renal pathologies of GSD1a that occur with aging.
58
59
60
61
62
63
64
65

1 **Compliance with Ethics Guidelines:**
2
3
4

5
6 **Animal rights**
7

8 All of the procedures were performed in accordance with the principles and guidelines
9 established by the European Convention for the Protection of Laboratory Animals. The
10 animal care committee of University Lyon 1 approved all of the experiments.
11
12
13
14
15
16
17

18 **Details of the contributions of individual authors**
19

20 Fabienne Rajas is the PI of this study. Julie Clar is a PhD student who characterized the renal
21 GSD1a mouse model. Amandine Gautier-Stein is a researcher who characterized glucose
22 transport by the intestine. Gilles Mithieux is the director of the lab.
23
24
25
26
27
28
29

30 **Competing Interests**
31

32 Fabienne Rajas, Julie Clar, Amandine Gautier-Stein and Gilles Mithieux declare that they
33 have no conflict of interest.
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5 Glycogen storage disease type 1 (GSD1) is a rare metabolic disorder characterized by
6
7 the absence of endogenous glucose production, leading to severe hypoglycemia following a
8
9 short fast (Chou et al. 2010; Froissart et al. 2011). This is caused by a deficiency of glucose-6
10
11 phosphatase (G6Pase), which is an enzyme complex involving the glucose-6 phosphate
12
13 translocase subunit (G6PT, encoded by *SLC37A4*) and the G6Pase catalytic subunit, encoded
14
15 by *G6PCI* (Soty et al. 2012) (Figure 1). Patients with GSD type 1a (GSD1a) represent 80%
16
17 of all GSD1 cases and have a G6PC deficiency, whereas patients with GSD type 1b (GSD1b)
18
19 have a G6PT defect. While G6PT is ubiquitously expressed, G6PC is expressed only in the
20
21 liver, kidneys and intestine (Rajas et al. 1999; Mithieux et al. 2004b; Rajas et al.
22
23 2007a)(Mithieux et al. 2004b; Rajas et al. 2007b). Both GSD1a and GSD1b patients show
24
25 broadly similar symptoms, including severe hypoglycemia in the post-absorptive state,
26
27 hyperlipidemia, hypercholesterolemia, hyperuricemia and lactic acidemia. G6Pase deficiency
28
29 leads to the accumulation of glycogen and triglycerides in the liver and kidneys. This results
30
31 in marked hepatomegaly, nephromegaly and hepatic steatosis. In addition, patients with
32
33 GSD1b present severe infectious complications, due to neutropenia and neutrophil and
34
35 monocyte functional defects.

36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

Until today, there is no cure for GSD1. In the past, many patients with GSD1 did not survive infancy and childhood. Since the eighties, life expectancy of patients with GSD1 has been considerably improved by stringent dietary treatment (Rake et al. 2002a; Heller et al. 2008). Frequent meals combined with uncooked cornstarch (during the day- and/or night-time) or gastric drip-feeding allow patients with GSD1 to avoid hypoglycemia and lactic acidemia. Despite the intensive dietary treatment, hepatic, renal and intestinal complications arise with aging (Di Rocco et al. 2008; Reddy et al. 2009). Thus a large proportion of patients

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

older than 20 years show hepatic tumors and/or progressive chronic renal disease, which are the leading causes of morbidity in GSD1 patients with aging. The first hepatic tumors generally appear during adolescence and are mainly classified as hepatocellular adenomas (HCA), which can transform into hepatocellular carcinomas (HCC) (Rake et al. 2002b; Franco et al. 2005; Wang et al. 2011; Calderaro et al. 2013). Tumor resection or liver transplantation are recommended if the tumors are associated with serious compression or hemorrhage, or show signs of malignant transformation into HCC (Rake et al. 2002a; Reddy et al. 2009). The first symptoms of renal disease are hyperperfusion and hyperfiltration and generally appear from childhood (Martens et al. 2009). Almost all adult patients show albuminuria and more than 50% present proteinuria (Martens et al. 2009). Finally, renal disease can slowly progress into renal failure that requires renal dialysis or transplantation (Rake et al. 2002a).

In order to investigate the onset of long-term pathologies developed by patients with GSD1a and to evaluate potential treatment strategies, we recently developed new mouse models of GSD1a that exclusively target G6PC deletion in the liver, kidneys or intestine (Mutel et al. 2011a; Penhoat et al. 2011; Clar et al. 2014). It is noteworthy that these tissue-specific G6PC knockout mice have normal life expectancy, whereas total G6PC knockout mouse and canine models die rapidly after weaning in the absence of intensive dietary therapy. In this review, we propose an overview of these new murine GSD1a models with tissue-specific *G6pc* deletions.

The canine and mouse models of total *G6pc* deficiency

Until now, the understanding of the biochemical bases of GSD1a and the evaluation of gene therapy approaches to correct *G6pc* deficiency were performed in two animal models of GSD1a. Both of these animal models are physiologically similar to humans in regard of the

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

glucose-6 phosphate metabolism. After the isolation of the *G6pc* gene, Dr. Janice Chou created the *G6pc* knockout mouse in 1993 (Lei et al. 1996). These mice present low birth weight, develop quickly severe and unremitting hypoglycemia quickly and gradually display pronounced increases in serum cholesterol and triglyceride levels (Table 1). However, they do not typically manifest lactic acidemia. Moreover, only 60% of mice treated with glucose injections, glucose-fortified water and food supplementation survive through weaning and have a life expectancy of about six months. **Although all *G6pc* knockout mice developed hepatomegaly and steatosis (Table 1), no hepatic tumors have been reported, even in mice as old as 6 months (Salganik et al. 2009). These mice exhibited nephromegaly as well (Table 1). Nevertheless, the renal pathology has been characterized only in an early stage (in 6-week-old KO mice) (Yiu et al. 2008).** A GSD1a canine model (Maltese breed) carrying a natural *G6pc* mutation was identified and used to characterize the disease as well (Kishnani et al. 1997). The Maltese breed is small in size and exhibits low survival rate of newborns. A second canine model was obtained by crossbreeding a carrier Maltese with beagles (Kishnani et al. 2001). Both canine models manifest all of the typical symptoms of the human disorder, including hyperlactacidemia, but they did not prove useful for studying the long-term complications of the GSD1a. Indeed, the dietary therapies used to maintain GSD1a animals viable have not yet been sufficiently refined to prevent premature death of these animals from hypoglycemia.

Rationale underlying the generation of organ-specific *G6pc* deficient mice.

The maintenance of blood glucose levels within a narrow range (about 5.5 mmol/L) is a critical physiological function. Although the liver is the main glucose-producing organ in the post-absorptive state *via* glycogenolysis, renal and intestinal glucose productions play a major role in maintaining normoglycemia during long fasting periods (Ekberg et al. 1999;

1 Gerich et al. 2001; Croset et al. 2001; Mithieux et al. 2004a). We hypothesized that **total**
2 inactivation of *G6pc* in only one gluconeogenic tissue would not be lethal due to the
3
4 compensatory **induction of glucose** production by the two non-targeted tissues. To delete the
5
6 G6Pase activity, we targeted the excision of *G6pc* exon 3, by using B6.G6pc^{ex3lox/ex3lox} mice.
7
8 We crossed B6.G6pc^{ex3lox/ex3lox} mice with mice expressing a CRE recombinase under a tissue-
9
10 specific promoter to target the *G6pc* deletion in the liver, kidney or intestine specifically. As
11
12 endogenous glucose production, especially in the liver, is critical during the neonatal period
13
14 because of the low content of glucose in milk (Girard et al. 1992; Chatelain et al. 1998), we
15
16 chose to induce *G6pc* deletion in adult mice by using a CRE^{ERT2} inducible by tamoxifen. The
17
18 CRE^{ERT2} is a recombinant CRE, fused to a mutated ligand-binding protein of the estrogen,
19
20 resulting in a tamoxifen-dependent CRE. In order to induce *G6pc* deletion, adult mice are
21
22 treated daily with 1 mg of tamoxifen for 5 consecutive days.
23
24
25
26
27
28
29
30

31 **Liver *G6pc* knockout mice.**

32
33
34 Hepatic *G6pc* deletion was targeted by crossing B6.G6pc^{ex3lox/ex3lox} mice with
35
36 B6.SA^{CREERT2/w} mice, which expressed the CRE^{ERT2} under the liver-specific serum albumin
37
38 promoter. Rapidly (10 days) after tamoxifen treatment, hepatic G6Pase activity was
39
40 undetectable in liver-specific *G6pc* knockout (L.G6pc^{-/-}) mice (Mutel et al. 2011a). As
41
42 expected, L.G6pc^{-/-} mice had normal life expectancy and showed normal blood glucose
43
44 during fed state (Table 1). Interestingly, L.G6pc^{-/-} mice did not exhibit marked hypoglycemia
45
46 during long fasting periods thanks to the induction of extrahepatic glucose production (Mutel
47
48 et al. 2011b; Penhoat et al. 2014). The livers of L.G6pc^{-/-} mice were rapidly enlarged, due to
49
50 the accumulation of glycogen and triglycerides (Table 1). In parallel, there was a rapid
51
52 increase in plasma triglyceride, cholesterol, uric acid, and lactic acid levels (Table 1).
53
54
55
56
57
58 However, these plasmatic parameters (except for cholesterol) improved after 6 months, as
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

observed in total *G6pc* knockout mice, which survive weaning thanks to a diet therapy (Salganik et al. 2009). We suggested that a satisfying blood glucose control could explain this amelioration. This is also in line with the observation that metabolic control is easier in adult patients with GSD1a, than during infancy or childhood.

Contrary to plasmatic parameters, glycogen storages were still elevated after 18 months of *G6pc* deletion. It is noteworthy that the accumulation of triglycerides increased with time. This could be linked to the late development of hepatocellular adenomas observed in L.*G6pc*^{-/-} mice fed a standard diet. The first millimetric nodules were detected by magnetic resonance imaging after 9 months of *G6pc* deletion in about 20% of L.*G6pc*^{-/-} mice. Most of them developed multiple nodules of about 1 to 10 mm in diameter after 18 months of *G6pc* deletion. Most of these tumors were HCA, but some L.*G6pc*^{-/-} livers (20%) presented dysplasia as well (Mutel et al. 2011a). **In addition, about 5-10% of L.*G6pc*^{-/-} mice fed a standard diet developed HCC (unpublished data).** It is important to note that the development of tumors appeared rather late, while liver steatosis tended to worsen. Moreover, our recent data show that the development of hepatic tumors in L.*G6pc*^{-/-} mice is enhanced by a high fat enriched diet (to be published). To propose therapeutic strategies or to update the dietary advices, it is now important to determine the molecular mechanisms involved in tumor development and to understand how is tumorigenesis influenced by the hepatic metabolism. Thus L.*G6pc*^{-/-} mice would be a unique model that could allow us to answer these questions and to study the effect of diet on the development of HCA and HCC.

Kidney *G6pc* knockout mice.

Renal *G6pc* deletion was targeted by crossing B6.*G6pc*^{ex3lox/ex3lox} mice with B6.Kap^{CREERT2/w} mice, which expressed the CRE^{ERT2} under the kidney androgen-regulated protein (Kap) promoter (Clar et al. 2014). This resulted in a partial lost (50% of inhibition) of

1 G6Pase activity after tamoxifen treatment of adult male mice. Immunohistological
2 observations of K-G6pc^{-/-} kidneys revealed a lower and heterogeneous staining of G6PC in
3
4 the cortex with the presence of only a few G6PC-positive cells identified in the proximal
5
6 convoluted tubules. Female B6.G6pc^{ex3lox/ex3lox}.Kap^{CREERT2/w} mice have to be treated with both
7
8 testosterone and tamoxifen to induce *G6pc* deletion. As expected, K-G6pc^{-/-} mice did not
9
10 suffer from hypoglycemia and did not require diet treatment. This is partially due to the
11
12 residual renal G6Pase activity and to a compensatory induction of hepatic G6Pase activity
13
14 (Clar et al. 2014). It is noteworthy that K-G6pc^{-/-} mice showed an early stage nephropathy i.e.
15
16 microalbuminuria and a partial electrolyte imbalance, after 6 months of *G6pc* deletion (Table
17
18 1) (Clar et al. 2014). However, they did not develop proteinuria, kidney fibrosis and
19
20 nephrolithiasis. On a molecular level, the accumulation of glucose-6 phosphate activated the
21
22 *de novo* lipogenesis pathway. The slight accumulation of triglycerides observed in the K-
23
24 G6pc^{-/-} kidney by red Sudan staining was sufficient to activate the renin-angiotensin system,
25
26 which was associated with an increased expression of the transforming growth factor β 1
27
28 (TGF β 1). This led to partial epithelial-mesenchymal transition (EMT)-like changes,
29
30 highlighted by a decrease of epithelial marker expression (e.g. E-cadherin, tight junction ZO-
31
32 1) and an overexpression of the mesenchymal marker fibronectin. Podocyte injury
33
34 characterized by a decrease in podocin, synaptopodin and podocalyxin expression was
35
36 observed in K-G6pc^{-/-} kidneys. However, no modifications in the thickness of basement
37
38 membrane or glomerulosclerosis were observed, suggesting that K-G6pc^{-/-} kidneys showed an
39
40 early stage of EMT only after 6 months of *G6pc* deletion (Clar et al. 2014).
41
42
43
44
45
46
47
48
49
50

51 The characterization of renal K-G6pc^{-/-} metabolism allowed us to highlight similarities
52
53 of molecular pathways involved in the development of EMT in both GSD1 and diabetes
54
55 (Mundy and Lee 2002; Rajas et al. 2013). Indeed, the accumulation of lipids or lipid
56
57 derivatives leads to the activation of renin- angiotensin system and subsequently of the
58
59
60
61
62
63
64
65

1 TGFβ1 pathway. The presence of lipid deposits in the renal cortex of two GSD1 patients with
2 proteinuria was reported in only one study (Obara et al. 1993). As opposed to diabetic
3 nephropathy, renal lipid accumulation in K-G6pc^{-/-} mice did not seem to be mediated by the
4 transcriptional factors SREBP1 (sterol regulatory element-binding protein), but rather by
5 ChREBP (carbohydrate-responsive element binding protein) (Clar et al. 2014). Compared to
6 the liver, little is known about lipid metabolism and lipid deposits in the kidney. The K-G6pc^{-/-}
7 mouse model is therefore a unique tool that can be used to decipher the role of lipids,
8 compared to glycogen accumulation, in the development of nephropathy.
9
10
11
12
13
14
15
16
17
18
19
20
21

22 **Intestinal *G6pc* knockout mice.**

23
24 Until now, intestinal symptoms were often underestimated in GSD1a patients, when
25 compared to GSD1b in whom they appear more serious (Visser et al. 2002). However, some
26 studies reported that GSD1a patients might also suffer from intermittent diarrhea due to
27 entero-proctitis (Fine et al. 1969; Milla et al. 1978; Rake et al. 2002b). An abnormal
28 accumulation of glycogen has also been reported in the intestine of GSD1a patients (Field et
29 al. 1965).
30
31
32
33
34
35
36
37
38

39 Intestinal *G6pc* deletion was targeted by crossing B6.G6pc^{ex3lox/ex3lox} mice with
40 B6.Villin^{CREERT2/w} mice, which express the CRE^{ERT2} under the Villin promoter (Penhoat et al.
41 2011). The specific deletion of *G6pc* in the intestine of I-G6pc^{-/-} mice was persistent for more
42 than one year. During the first year after *G6pc* gene deletion, I-G6pc^{-/-} mice exhibited a
43 growth rate similar to that of wild-type mice. Meanwhile, no diarrhea or abnormal
44 consistency of the stools was observed in any I-G6pc^{-/-} mice, compared to wild-type mice.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 influence intestine metabolism. Hence, we cannot exclude that some adverse effects could
2 appear with time according to the diet composition. This hypothesis will be assessed in the I-
3
4 $G6pc^{-/-}$ mice in the near future.
5
6

7 For example, the ingestion of too much sugar, including uncooked starched, in patients
8 with GSD1 could lead to bacterial proliferation. A bacterial overgrowth has been documented
9 in a GSD1b patient by using a hydrogen breath test (Santer et al. 2003). In the latter study, the
10 authors suggested that the absence of a functional G6Pase might induce carbohydrate
11 malabsorption by the intestine and promote bacterial overgrowth. Indeed, G6Pase has been
12 suggested to be involved in intestinal transepithelial glucose transport. The transepithelial
13 glucose transport in the intestine is thought to mainly involve the sodium-dependent glucose
14 cotransporter SGLT1 at the apical membrane and the glucose transporter GLUT2 at the basal
15 membrane. However, glucose absorption, challenged by an oral glucose test, is similar in
16 $Glut2^{-/-}$ mice and wild-type mice, suggesting the existence of another intestinal glucose
17 transport pathway (Stümpel et al. 2001). The absorption of glucose measured in isolated
18 perfused intestine and liver of $Glut2^{-/-}$ mice is inhibited by S4048, a specific inhibitor of
19 G6PT. In the absence of GLUT2, a step of phosphorylation (by hexokinase) and hydrolysis
20 (by G6Pase) seems thus needed for glucose transport through the intestine. This would be
21 consistent with a high hexokinase activity in the intestine, 10-fold greater than the reported
22 maximal flux of glucose through glycolysis (Newsholme and Carrié 1994). Finally, the gut
23 microbiota fermentation is tightly coupled to intestinal metabolism (De Vadder et al. 2014). I-
24 $G6pc^{-/-}$ mice are thus a unique model that can be used to study the precise role of intestinal
25 glucose absorption and metabolism in the etiology of intestinal symptoms of GSD1 patients.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Lessons from the tissue-specific *G6pc* knockout mouse models

1
2 The usefulness of these new animal models of GSD1a is based primarily on the fact
3
4 that these animals are viable. They permitted the characterization of the development of
5
6 hepatic tumors and nephropathy during several months. These results suggest that the hepatic
7
8 or renal *G6pc* deficiency *per se* is sufficient to induce independently the development of
9
10 hepatic or renal pathology, respectively. This highlighted the fact that the development of
11
12 nephropathy is independent of the liver-derived metabolic changes. This was an important
13
14 finding in the context of the pathophysiological concept of the GSD1 disease as a whole, but
15
16 also in terms of transplantation or gene therapy. Kidney transplantation is performed in the
17
18 case of severe renal failure, but this does not correct hypoglycemia (Emmett and Narins
19
20 1978). In contrast, liver transplantation allows a correction of the glycemia and all liver-
21
22 related biochemical abnormalities, but any beneficial effects on the kidney remain to be
23
24 proven (Faivre et al. 1999; Labrune 2002; Davis and Weinstein 2008; Reddy et al. 2009).
25
26 This finding suggests that clinicians should discuss the long-term benefits and possible
27
28 convenience of a double kidney/liver transplantation for GSD1a patients, who have developed
29
30 multiple liver HCAs or HCCs or renal failure.
31
32
33
34
35
36
37

38
39 Concerning gene therapy, it was already shown that the rescue of G6Pase activity in
40
41 the liver by using an AAV8 recombinant vector allowed total *G6pc* KO mice to maintain
42
43 normal blood glucose levels, but it did not prevent the development of the nephropathy (Yiu
44
45 et al. 2010; Luo et al. 2011). As AAV1 or AAV2/9 vectors are able to transduce both the liver
46
47 and the kidney, these vectors seem to be a better choice for GSD1a gene therapy (Ghosh et al.
48
49 2006; Luo et al. 2011). Thus, the hepatic and renal $G6pc^{-/-}$ mouse models will be useful to test
50
51 the efficiency and the safety of gene therapy. In addition, $L.G6pc^{-/-}$ mice could be used to test
52
53 drugs targeting the development of hepatic tumors. Interestingly, the molecular mechanisms
54
55 involved in GSD1a nephropathy are very similar to those of diabetic patients (Mundy and Lee
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2002; Rajas et al. 2013). Therefore, K.G6pc^{-/-} mice could be a useful tool in future studies of the pharmacological treatment of EMT and/or kidney failure developed by patients with either GSD1 or diabetes.

Finally, these mice should allow us to improve the strategies of treatment on a nutritional point of view. Indeed, preliminary results showed that diet could greatly influence the development of hepatic tumors in L.G6pc^{-/-} mice (to be published). Moreover, until now, dietary guidelines were only based on biochemical knowledge and vary greatly between pediatricians. For example, some clinicians prohibit entirely the consumption of fruits, juice, dairy products and sweets. Others only limit the consumption of these products, in order to restrain the intake of fructose, lactose and sucrose. To provide scientific evidence, L.G6pc^{-/-} and K.G6pc^{-/-} mice will be useful to analyze the effect of diet on the development of hepatic tumors and nephropathy, respectively. Meanwhile, it is important to remind teenagers and adults to follow strictly their diet, even if they already exhibit a good metabolic control, in order to delay or avoid the development of the GSD1 complications.

Legend of figure:

Figure 1: Schematic representation of the glucose-6 phosphatase. This enzyme is composed of two subunits localized in the membrane of the endoplasmic reticulum: the transport subunit (G6PT) and the catalytic subunit (G6PC). The G6PT subunit is ubiquitously expressed, whereas the G6PC is expressed only in the liver, kidneys and intestine. The mutations of G6PC are responsible for GSD type 1a and the mutations of G6PT are responsible for GSD type 1b. Images were made with Servier Medical Art illustrations.

1
2
3
4
5
6
7
8
9
10
Financial support: This work was supported by research grants from the “Agence Nationale
de la Recherche” (ANR-07-MRAR-011-01 and ANR11-BSV1-009-01) and the “Association
Francophone des Glycogénoses”.

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
References:

34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
Calderaro J, Labrune P, Morcrette G, et al. (2013) Molecular characterization of
hepatocellular adenomas developed in patients with glycogen storage disease type I. *J Hepatol*
58:350–357. doi: 10.1016/j.jhep.2012.09.030

Chatelain F, Pégorier JP, Minassian C, et al. (1998) Development and regulation of
glucose-6-phosphatase gene expression in rat liver, intestine, and kidney: in vivo and in vitro
studies in cultured fetal hepatocytes. *Diabetes* 47:882–889.

Chou JY, Jun HS, Mansfield BC (2010) Glycogen storage disease type I and G6Pase- β
deficiency: etiology and therapy. *Nat Rev Endocrinol* 6:676–688. doi:
10.1038/nrendo.2010.189

Clar J, Gri B, Calderaro J, et al. (2014) Targeted deletion of kidney glucose-6
phosphatase leads to nephropathy. *Kidney Int.* doi: 10.1038/ki.2014.102

Croset M, Rajas F, Zitoun C, et al. (2001) Rat small intestine is an insulin-sensitive
gluconeogenic organ. *Diabetes* 50:740–746.

Davis MK, Weinstein DA (2008) Liver transplantation in children with glycogen storage
disease: controversies and evaluation of the risk/benefit of this procedure. *Pediatr Transplant*
12:137–145. doi: 10.1111/j.1399-3046.2007.00803.x

De Vadder F, Kovatcheva-Datchary P, Goncalves D, et al. (2014) Microbiota-generated
metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* 156:84–96. doi:
10.1016/j.cell.2013.12.016

Di Rocco M, Calevo MG, Taro' M, et al. (2008) Hepatocellular adenoma and metabolic

1 balance in patients with type Ia glycogen storage disease. *Mol Genet Metab* 93:398–402. doi:
2 10.1016/j.ymgme.2007.10.134
3

4 Ekberg K, Landau BR, Wajngot A, et al. (1999) Contributions by kidney and liver to
5 glucose production in the postabsorptive state and after 60 h of fasting. *Diabetes* 48:292–298.
6

7 Emmett M, Narins RG (1978) Renal transplantation in type 1 glycogenosis. Failure to
8 improve glucose metabolism. *JAMA J Am Med Assoc* 239:1642–1644.
9

10 Faivre L, Houssin D, Valayer J, et al. (1999) Long-term outcome of liver transplantation
11 in patients with glycogen storage disease type Ia. *J Inherit Metab Dis* 22:723–732.
12

13 Field JB, Epstein S, Egan T (1965) Studies in glycogen storage diseases. I. Intestinal
14 glucose-6 phosphatase activity in patients with Von Gierke's disease and their parents. *J Clin*
15 *Invest* 44:1240–1247. doi: 10.1172/JCI105230
16

17 Fine RN, Kogut MD, Donnell GN (1969) Intestinal absorption in type I glycogen storage
18 disease. *J Pediatr* 75:632–635.
19

20 Franco LM, Krishnamurthy V, Bali D, et al. (2005) Hepatocellular carcinoma in
21 glycogen storage disease type Ia: a case series. *J Inherit Metab Dis* 28:153–162. doi:
22 10.1007/s10545-005-7500-2
23

24 Froissart R, Piraud M, Boudjemline AM, et al. (2011) Glucose-6-phosphatase deficiency.
25 *Orphanet J Rare Dis* 6:27. doi: 10.1186/1750-1172-6-27
26

27 Gerich JE, Meyer C, Woerle HJ, Stumvoll M (2001) Renal gluconeogenesis: its
28 importance in human glucose homeostasis. *Diabetes Care* 24:382–391.
29

30 Ghosh A, Allamarvdasht M, Pan C-J, et al. (2006) Long-term correction of murine
31 glycogen storage disease type Ia by recombinant adeno-associated virus-1-mediated gene
32 transfer. *Gene Ther* 13:321–329. doi: 10.1038/sj.gt.3302650
33

34 Girard J, Ferré P, Pégrier JP, Duée PH (1992) Adaptations of glucose and fatty acid
35 metabolism during perinatal period and suckling-weaning transition. *Physiol Rev* 72:507–
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

562.

1
2 Heller S, Worona L, Consuelo A (2008) Nutritional therapy for glycogen storage
3 diseases. *J Pediatr Gastroenterol Nutr* 47 Suppl 1:S15–21. doi:
4
5 10.1097/MPG.0b013e3181818ea5
6
7

8
9 Kim SY, Weinstein DA, Starost MF, et al. (2008) Necrotic foci, elevated chemokines and
10 infiltrating neutrophils in the liver of glycogen storage disease type Ia. *J Hepatol* 48:479–485.
11
12 doi: 10.1016/j.jhep.2007.11.014
13
14

15
16 Kishnani PS, Bao Y, Wu JY, et al. (1997) Isolation and nucleotide sequence of canine
17 glucose-6-phosphatase mRNA: identification of mutation in puppies with glycogen storage
18
19 disease type Ia. *Biochem Mol Med* 61:168–177.
20
21
22

23
24 Kishnani PS, Faulkner E, VanCamp S, et al. (2001) Canine model and genomic structural
25 organization of glycogen storage disease type Ia (GSD Ia). *Vet Pathol* 38:83–91.
26
27

28
29 Labrune P (2002) Glycogen storage disease type I: indications for liver and/or kidney
30 transplantation. *Eur J Pediatr* 161 Suppl 1:S53–55. doi: 10.1007/s00431-002-1004-y
31
32

33
34 Lei KJ, Chen H, Pan CJ, et al. (1996) Glucose-6-phosphatase dependent substrate
35 transport in the glycogen storage disease type-Ia mouse. *Nat Genet* 13:203–209. doi:
36
37 10.1038/ng0696-203
38
39

40
41 Luo X, Hall G, Li S, et al. (2011) Hepatorenal correction in murine glycogen storage
42 disease type I with a double-stranded adeno-associated virus vector. *Mol Ther J Am Soc Gene*
43
44 *Ther* 19:1961–1970. doi: 10.1038/mt.2011.126
45
46

47
48 Martens DHJ, Rake JP, Navis G, et al. (2009) Renal function in glycogen storage disease
49 type I, natural course, and renopreservative effects of ACE inhibition. *Clin J Am Soc Nephrol*
50
51 *CJASN* 4:1741–1746. doi: 10.2215/CJN.00050109
52
53

54
55 Milla PJ, Atherton DA, Leonard JV, et al. (1978) Disordered intestinal function in
56
57 glycogen storage disease. *J Inherit Metab Dis* 1:155–157.
58
59
60
61
62
63
64
65

1 Mithieux G, Bady I, Gautier A, et al. (2004a) Induction of control genes in intestinal
2 gluconeogenesis is sequential during fasting and maximal in diabetes. *Am J Physiol*
3
4 *Endocrinol Metab* 286:E370–375. doi: 10.1152/ajpendo.00299.2003
5
6

7 Mithieux G, Rajas F, Gautier-Stein A (2004b) A novel role for glucose 6-phosphatase in
8
9 the small intestine in the control of glucose homeostasis. *J Biol Chem* 279:44231–44234. doi:
10
11 10.1074/jbc.R400011200
12
13

14 Mundy HR, Lee PJ (2002) Glycogenesis type I and diabetes mellitus: a common
15
16 mechanism for renal dysfunction? *Med Hypotheses* 59:110–114.
17
18

19 Mutel E, Abdul-Wahed A, Ramamonjisoa N, et al. (2011a) Targeted deletion of liver
20
21 glucose-6 phosphatase mimics glycogen storage disease type 1a including development of
22
23 multiple adenomas. *J Hepatol* 54:529–537. doi: 10.1016/j.jhep.2010.08.014
24
25

26 Mutel E, Gautier-Stein A, Abdul-Wahed A, et al. (2011b) Control of blood glucose in the
27
28 absence of hepatic glucose production during prolonged fasting in mice: induction of renal
29
30 and intestinal gluconeogenesis by glucagon. *Diabetes* 60:3121–3131. doi: 10.2337/db11-0571
31
32
33

34 Newsholme EA, Carrié AL (1994) Quantitative aspects of glucose and glutamine
35
36 metabolism by intestinal cells. *Gut* 35:S13–17.
37
38

39 Obara K, Saito T, Sato H, et al. (1993) Renal histology in two adult patients with type I
40
41 glycogen storage disease. *Clin Nephrol* 39:59–64.
42
43

44 Penhoat A, Fayard L, Stefanutti A, et al. (2014) Intestinal gluconeogenesis is crucial to
45
46 maintain a physiological fasting glycemia in the absence of hepatic glucose production in
47
48 mice. *Metabolism* 63:104–111. doi: 10.1016/j.metabol.2013.09.005
49
50

51 Penhoat A, Mutel E, Amigo-Correig M, et al. (2011) Protein-induced satiety is abolished
52
53 in the absence of intestinal gluconeogenesis. *Physiol Behav* 105:89–93. doi:
54
55 10.1016/j.physbeh.2011.03.012
56
57

58 Rajas F, Bruni N, Montano S, et al. (1999) The glucose-6 phosphatase gene is expressed
59
60
61
62
63
64
65

1
2 in human and rat small intestine: regulation of expression in fasted and diabetic rats.
3 Gastroenterology 117:132–139.

4
5 Rajas F, Jourdan-Pineau H, Stefanutti A, et al. (2007a) Immunocytochemical localization
6
7 of glucose 6-phosphatase and cytosolic phosphoenolpyruvate carboxykinase in gluconeogenic
8
9 tissues reveals unsuspected metabolic zonation. Histochem Cell Biol 127:555–565. doi:
10
11 10.1007/s00418-006-0263-5
12

13
14 Rajas F, Jourdan-Pineau H, Stefanutti A, et al. (2007b) Immunocytochemical localization
15
16 of glucose 6-phosphatase and cytosolic phosphoenolpyruvate carboxykinase in gluconeogenic
17
18 tissues reveals unsuspected metabolic zonation. Histochem Cell Biol 127:555–565. doi:
19
20 10.1007/s00418-006-0263-5
21
22

23
24 Rajas F, Labrune P, Mithieux G (2013) Glycogen storage disease type 1 and diabetes:
25
26 Learning by comparing and contrasting the two disorders. Diabetes Metab. doi:
27
28 10.1016/j.diabet.2013.03.002
29
30

31
32 Rake JP, Visser G, Labrune P, et al. (2002a) Guidelines for management of glycogen
33
34 storage disease type I - European Study on Glycogen Storage Disease Type I (ESGSD I). Eur
35
36 J Pediatr 161 Suppl 1:S112–119. doi: 10.1007/s00431-002-1016-7
37

38
39 Rake JP, Visser G, Labrune P, et al. (2002b) Glycogen storage disease type I: diagnosis,
40
41 management, clinical course and outcome. Results of the European Study on Glycogen
42
43 Storage Disease Type I (ESGSD I). Eur J Pediatr 161 Suppl 1:S20–34. doi: 10.1007/s00431-
44
45 002-0999-4
46
47

48
49 Reddy SK, Austin SL, Spencer-Manzon M, et al. (2009) Liver transplantation for
50
51 glycogen storage disease type Ia. J Hepatol 51:483–490. doi: 10.1016/j.jhep.2009.05.026
52

53
54 Salganik SV, Weinstein DA, Shupe TD, et al. (2009) A detailed characterization of the
55
56 adult mouse model of glycogen storage disease Ia. Lab Invest J Tech Methods Pathol
57
58 89:1032–1042. doi: 10.1038/labinvest.2009.64
59
60
61
62
63
64
65

1 Santer R, Hillebrand G, Steinmann B, Schaub J (2003) Intestinal glucose transport:
2 evidence for a membrane traffic-based pathway in humans. *Gastroenterology* 124:34–39. doi:
3
4 10.1053/gast.2003.50009
5
6

7 Soty M, Chilloux J, Casteras S, et al. (2012) New insights into the organisation and
8
9 intracellular localisation of the two subunits of glucose-6-phosphatase. *Biochimie* 94:695–
10
11 703. doi: 10.1016/j.biochi.2011.09.022
12
13

14 Stümpel F, Burcelin R, Jungermann K, Thorens B (2001) Normal kinetics of intestinal
15
16 glucose absorption in the absence of GLUT2: evidence for a transport pathway requiring
17
18 glucose phosphorylation and transfer into the endoplasmic reticulum. *Proc Natl Acad Sci U S*
19
20 *A* 98:11330–11335. doi: 10.1073/pnas.211357698
21
22
23

24 Visser G, Rake JP, Kokke FTM, et al. (2002) Intestinal function in glycogen storage
25
26 disease type I. *J Inherit Metab Dis* 25:261–267.
27
28

29 Wang DQ, Fiske LM, Carreras CT, Weinstein DA (2011) Natural history of
30
31 hepatocellular adenoma formation in glycogen storage disease type I. *J Pediatr* 159:442–446.
32
33 doi: 10.1016/j.jpeds.2011.02.031
34
35

36 Yiu WH, Lee YM, Peng W-T, et al. (2010) Complete normalization of hepatic G6PC
37
38 deficiency in murine glycogen storage disease type Ia using gene therapy. *Mol Ther J Am Soc*
39
40 *Gene Ther* 18:1076–1084. doi: 10.1038/mt.2010.64
41
42
43

44 Yiu WH, Pan C-J, Ruef RA, et al. (2008) Angiotensin mediates renal fibrosis in the
45
46 nephropathy of glycogen storage disease type Ia. *Kidney Int* 73:716–723. doi:
47
48 10.1038/sj.ki.5002718
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Table 1. The characteristics of mouse models of GSD1a.

	Total G6pc ^{-/-} mice	Liver G6pc ^{-/-} mice	Kidney G6pc ^{-/-} mice	Intestinal G6pc ^{-/-} mice
Glycaemia	≈40-50mg/dL in the fed state	Normal in the fed state (≈150-200 mg/dL) 50-60 mg/dL at 6h of fasting	Normal in the fed state Normal at 6h of fasting (≈140 mg/dL)	Normal
Plasmatic parameters	Hypertriglyceridemia (≈1.8-2.0 g/L) Hypercholesterolemia (≈1.8 g/L) Hyperuricemia (≈25 mg/L) No or slight lactic acidemia	Hypertriglyceridemia (≈1.2 mg/L) Hypercholesterolemia (≈1.2 g/L) Hyperuricemia (≈25 mg/L) Lactic acidemia	Normal TG Normal cholesterol Hyperuricemia (≈11 mg/L) Normal lactic acid	Normal
Liver	Hepatomegaly (liver weight 10% BW) Steatosis	Hepatomegaly (liver weight: 8% of BW) Steatosis Late development of HCA*	Normal	Normal
Kidney	Nephromegaly (kidney weight: ≈3.5% BW)	Normal	Nephromegaly (kidney weight: ≈1.8% BW) Renal lipid accumulation Microalbuminuria Partial electrolyte imbalance	Normal
Intestine	Not determined	Normal	Normal	To be analyzed
Life expectancy	Need intensive glucose therapy or gene therapy to survive weaning	Normal	Over 12 months	Normal

The characteristics of 6-week-old total G6pc^{-/-} mice were described by the Chou's team (Kim et al. 2008) (Lei et al. 1996). The data of liver G6pc^{-/-} mice were obtained one month after G6pc deletion. *The first hepatic lesions were observed 9 months after G6pc deletion (Mutel et al. 2011a). It is noteworthy that plasmatic parameters ameliorate with age in both GSD1a mouse models and patients. The data of kidney G6pc^{-/-} mice were obtained 6 months after G6pc deletion (Clar et al. 2014). Intestinal G6pc^{-/-} mice were analyzed up to 12 months after G6pc deletion (unpublished data). BW: body weight. Normal range of plasmatic values in 6 week-old C57Bl/6J mice: TG: 0.55-0.6 g/L; cholesterol: 0.7-0.8 g/L; Uric acid: 6-8 mg/L; liver and kidney weights represent about 4% and 1% of BW, respectively.

Table
[Click here to download Table: Clar et al., Table 1.pdf](#)

	Total G6pc ^{-/-} mice	Liver G6pc ^{-/-} mice	Kidney G6pc ^{-/-} mice	Intestinal G6pc ^{-/-} mice
Glycaemia	≈40-50mg/dL in the fed state	Normal in the fed state (≈150-200 mg/dL) 50-60 mg/dL at 6h of fasting	Normal in the fed state Normal at 6h of fasting (≈140 mg/dL)	Normal
Plasmatic parameters	Hypertriglyceridemia (≈1.8-2.0 g/L) Hypercholesterolemia (≈1.8 g/L) Hyperuricemia (≈25 mg/L) No or slight lactic acidemia	Hypertriglyceridemia (≈1.2 mg/L) Hypercholesterolemia (≈1.2 g/L) Hyperuricemia (≈25 mg/L) Lactic acidemia	Normal TG Normal cholesterol Hyperuricemia (≈11 mg/L) Normal lactic acid	Normal
Liver	Hepatomegaly (liver weight 10% BW) Steatosis	Hepatomegaly (liver weight: 8% of BW) Steatosis Late development of HCA*	Normal	Normal
Kidney	Nephromegaly (kidney weight: ≈3.5% BW)	Normal	Nephromegaly (kidney weight: ≈1.8% BW) Renal lipid accumulation Microalbuminuria Partial electrolyte imbalance	Normal
Intestine	Not determined	Normal	Normal	To be analyzed
Life expectancy	Need intensive glucose therapy or gene therapy to survive weaning	Normal	Over 12 months	Normal

Figure 1
[Click here to download Figure: Rajas et al., Figure 1.pdf](#)

