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Functional invalidation of the autotaxin gene by a single amino acid mutation in mouse is lethal

Gilles Ferry[a], Adeline Giganti[a], Francis Cogé[a], Fabien Bertaux[b], Kader Thiam[b], Jean A. Boutin[a,*]

[a] Pharmacologie Moléculaire et Cellulaire, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy-sur-Seine, France
[b] genOway, Immeuble Chateaubriand, 181, Avenue Jean Jaures, 69362 Lyon Cedex 07, France

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Abstract **Autotaxin is a member of the phosphodiesterase family of enzymes, (NPP2). It is an important secreted protein found in conditioned medium from adipocytes. It also has a putative role in the metastatic process. Based on these observation, further validation of this potential target was necessary, apart from the classical biochemical ones. The construction of a knock out mouse strain for ATX was started. In this paper, we report the generation of a mouse line displaying an inactivated ATX gene product. The KO line was designed in order to generate a functional inactivation of the protein. In this respect, the threonine residue T210 was replaced by an alanine (T210A) leading to a catalytically inactive enzyme. If the experimental work was straight forward, we disappointedly discovered at the final stage that the breeding of heterozygous animals, ATX −/+, led to the generation of a Mendelian repartition of wild-type and heterozygous, but no homozygous were found, strongly suggesting that the ATX deletion is lethal at an early stage of the development. This was confirmed by statistical analysis. Although other reported the same lethality for attempted ATX−/− mice generation [van Meeteren, L.A., Ruurs, P., Stortelers, C., Bouwman, P., van Rooijen, M.A., Pradère, J.P., Pettit, T.R., Wakelam, M.J.O., Saulnier-Blache, J.S., Mummery, C.L., Moolenar, W.H. and Jonkers, J. (2006) Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development, Mol. Cell. Biol. 26, 5015–5022; Tanaka, M., Okudaira, S., Kishi, Y., Ohkawa, R., Isei, S., Ota, M., Noji, S., Yatomi, Y., Aoki, J., and Arai, H. (2006) Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysophosphatidic acid, J. Biol. Chem. 281, 25822–25830], they used more drastic multiple exon deletions in the ATX gene, while we chose a single point mutation. To our knowledge, the present work is the first showing such a lethality in any gene after a point mutation in an enzyme catalytic site.**
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Keywords: Mouse knock-out; Autotaxin; Cre-excision; Lysophospholipase D; Lyso-phosphatidic acid

1. Introduction

ATX is a lyso-phospholipase D enzyme originally described as a motility factor [1]. Since the discover, by others [2,3] and by us [4] that this enzyme catalyzes a lyso-phospholipase D (LPD) activity leading to the transformation of lyso-phosphatildylcholine into LPA, the role of this protein seemed to be central in some key metabolic deregulations (see Moolenaar for reviews [5,6]), particularly since it is over-expressed in the conditioned medium of adipocytes in culture [7]. ATX could be the main enzymatic source for the production of LPA, the role of which in metastasis has just been demonstrated [8]. Furthermore, it is known for quite some times that the LPA concentration is proportional to the metastasis progression, and therefore to cancer prognosis (see Jansen et al. [9] for complete references). Finally, even more direct evidences associated the expression of ATX with breast cancer invasiveness [10,11].

While making other attempts to provide more validation in term of involvement of this enzyme in diabesity [12], we attempted to knock-out the gene in mouse. This would have provide us with further validation towards the therapeutical use of this new target, beside biochemical [4] and pharmacological studies [13].

ATX has been described in the past as a motility factor [14]. Indeed, autotaxin was suspected to act through a putative receptor, before the discovery of its lyso-PLD catalytic activity. Hence, the presence of a RGD motif in the sequence pointed to the possibility that the protein itself might also act as a ligand or a partner to another protein. We therefore suspected that the deletion of a part of the protein might have deleterious effect on the mouse development. Since previous data evidenced that the point mutation inside the catalytic site (threonine 210 to alanine) leads to a catalytically inactive enzyme [15,16], we decided to reproduce this point mutation in vivo in order to circumvent any deleterious effect that the ATX gene classical disruption approach may have. Despite many difficulties in generating ATX targeted ES cells, we finally only succeeded in generating heterozygous mice with a single loss of ATX gene, whereas no homozygous mice were produced. This strongly suggests that the ATX $T > A^{210}$ mutation is lethal at the homozygous stage.

2. Materials and methods

2.1. Construction of the targeting vector

Targeting vector construction and knock-in strategy has been designed and performed by genOway (Lyon, France). Genomic clones containing the murine *Atx* locus were isolated from a 129S6/SvEvTac RPCI-22 BAC genomic library by using probe corresponding to the

*Corresponding author. Fax: +33 1 55 72 28 10.
E-mail address: jean.Boutin@fr.netgrs.com (J.A. Boutin).

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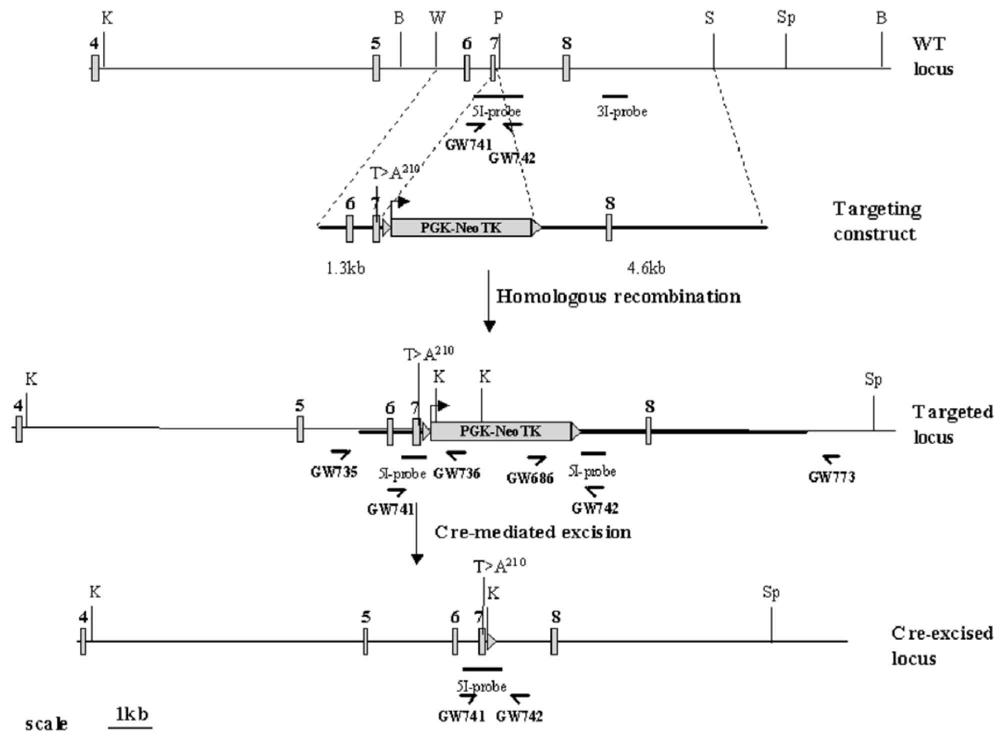

Fig. 1. Targeted mutation of the *Atx* gene produces a null mutation. The *Atx* locus (containing the exons 4–8) and the targeting construct (containing the neomycin Tk(PGK-*NeoTK*) cassette with flanking segments homologous to the locus) are shown in schematic format. The transcriptional orientation of neomycin-TK cassette and the *Atx* locus are delineated by *arrows.* The probe used in all the Southern blot analysis is a 1.0-kb fragment located in a region overlapping exon 7. B: BglII site; K: KnpI site; P: PacI site, S: SacI site, W: SwaI site. The arrow head corresponds to the LoxP site.

murine *Atx* exons 1 and 2: two BAC clones (130B15 and 287J20) containing *Atx* locus have been isolated. The genomic organization of the targeted locus was determined by subcloning BglII–SacI genomic fragment into the pZErO™-2 vector (Invitrogen, Carlsbad, CA). The 6.6-kb BglII–SacI genomic insert was sequenced and *Atx* sequence was generated. The genomic clone (containing introns V–VIII) was used to construct the targeting vector. Briefly, a 1.3-kb SwaI–PacI fragment comprising *Atx* exons 6 and 7 and a 4.6-kb PacI–SacI fragment located downstream of the *Atx* seventieth exon, were used to flank a NEO-tk cassette (*Lox*P site-PGK promoter-Neo-tk fusion cDNA-*Lox*P site) (as shown in Fig. 1); a negative (DTA) selection cassette was introduced at the 5′ of the short arm of homology. A $T > A^{210}$ point mutation was introduced into the *Atx* exon 7 using QuikChange® II site-directed mutagenesis kit (Stratagene, La Jolla, CA).

Furthermore, we cloned from 129S6/Sv mouse brain the ATX coding region into pcDNA3 vector and introduced by directed mutagenesis the $T > A^{210}$ mutation. We further used the two vectors, wild-type and mutated one, for expression in COS cells. As an internal control, we used a pcDNA3 vector containing the ATX sequence largely deleted from amino acids 202 to 363, which corresponds to the deletion of the whole catalytic site.

2.2. Screening of Atx targeted ES cell clones

AvrII-linearized targeting vector was transfected into 129SvPas ES cells (genOway, Lyon, France) according to genOway's modified electroporation procedures (i.e. 10^8 ES cells in presence of 100 μg of linearized plasmid, 260 V, 500 μF). Positive selection was started 48 h after electroporation, by addition of 200 μg/ml of G418 (150 μg/ml of active component, Life Technologies, Inc.). Three hundred and thirty-one resistant clones were isolated and amplified in 96-well plates. The duplicates of 96-well plates were made. The set of plates containing ES cell clones amplified on gelatin were screened by PCR and further confirmed by Southern blot. 5′ PCR screening conditions were: GW735 primer hybridizes the *Atx* intron 5 (5′-GGCGACAGCC-ACATTGAGTGACAC-3′), and GW736 primer is specific for the Neo-Tk selection cassette (5′-CGGTGGATGTGGAATGTGTGCG-3′). PCR conditions are 94 °C/5 min, 35 cycles of (92 °C/30 s, 62 °C/30 s, 68 °C/4 min 30 s) and then 68 °C/10 min, which results in a 2046-bp band for the mutated allele; PCR reaction is performed using Long Expand High Fidelity polymerase (Roche®) and reaction buffer 3. The 3′ PCR screening conditions were: GW686 primer is specific for the neomycin-tk selection cassette (5′-GGTGGAGAGGCTATTC-GGCTATGAC-3′), and GW773 primer is specific for the *Atx* intron 7 (5′-CTCCTGCCTCCACATTCCTTCCCTG-3′). PCR conditions are 94 °C/2 min, 35 cycles of (94 °C/30 s, 65 °C/30 s, 68 °C/6 min) and then 68 °C/7 min, which results in a 6096-bp band for the mutated allele; PCR reaction is performed using Long Expand High Fidelity polymerase (Roche®) and reaction buffer 3. Briefly, for Southern blot analysis, genomic DNA was digested with SpeI–KpnI and then hybridized with a 1.0-kb internal probe; *Atx*+/− clones give rise to an 11.3-kb wild-type signal and 8.1-kb + 5.3-kb targeted signals. The presence of the $T > A^{210}$ point mutation was confirmed by sequencing the 5′ PCR amplicon. Four clones (#4E6, 4G1, 3B4 and 3D1) were identified, by both PCR and Southern blot as targeted at the *Atx* locus (Fig. 2).

2.3. Generation of Chimera mice and breeding scheme

One floxed mutated *Atx* ES cell clone (namely #3B4) was microinjected into C57BL/6 blastocysts, and gave rise to male chimeras with a significant ES cell contribution (as determined by an Agouti coat color). After mating with C57Bl/6 females, germ line transmission was confirmed by the genotyping of tail DNA offsprings using PCR and Southern blot analysis. Floxed heterozygous animals were screened as described in previous chapter (Fig. 3). F1 male heterozygous animals were bred with C57/Bl6 CMV-Cre expressing female mice in order to remove the *Lox*P-flanked Neo cassette. Offsprings were genotyped by PCR and Southern blot in order to ensure the Neo cassette removal. PCR screening conditions are: the GW741 primer is specific for the 3′ region of intron 6 (5′-CCTACAT-GAGGCCTGTGTACCCTACAAAAG-3′), and the GW742 primer is specific for intron 7 (5′-GTCTGAGTTTCATCCCAGAACCCG-TATG-3′). PCR conditions are 94 °C/2 min, 35 cycles of (94 °C/30 s,

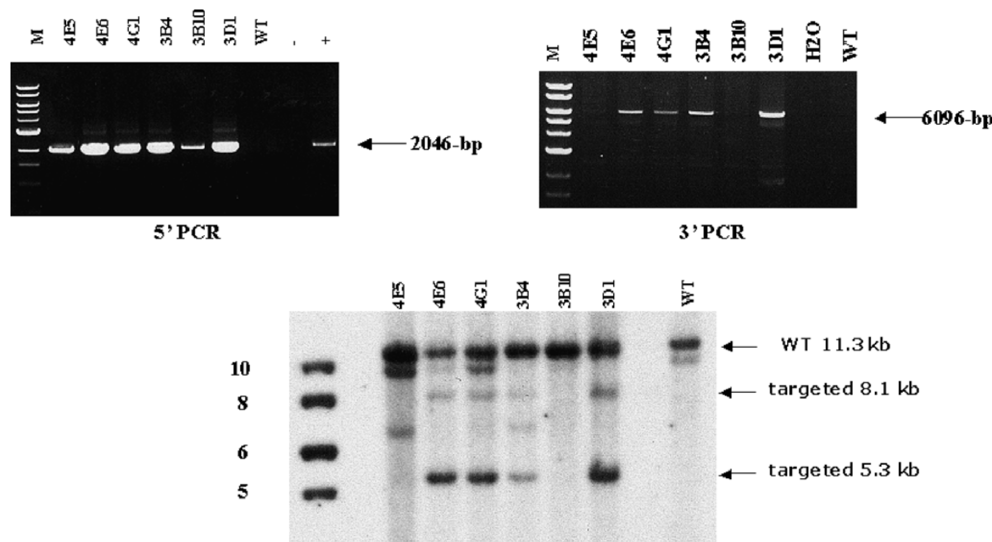

Fig. 2. PCR screening on 5′ and 3′ end of homologous recombination event. The PCR screening on 5′ and 3′ end of homologous recombination event is shown for ES cell clones #4E5, #4E6, #4G1, #3B4, #3B10 and #3D1. The 2046-bp and 6096-bp bands signify appropriated targeted disruption of m*Atx* locus on 5′ and 3′ end targeting event, respectively. Southern blot analysis of positive and wild-type ES cell clones. Digestion of genomic DNA with KpnI–SpeI resulted in the following diagnostic fragment: wild-type allele of 11.3-kb band, appropriate targeting of the*Atx* locus of 8.1- and 5.3-kb band.

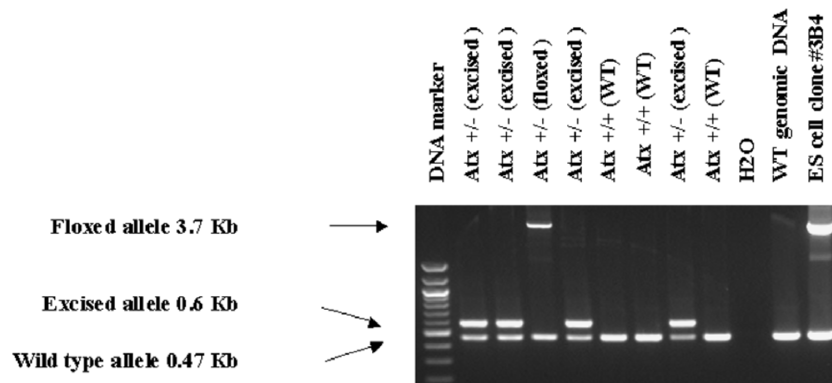

Fig. 3. PCR screening analysis of the offspring of *Atx*-mutated mice. The PCR screening analysis of the offspring of *Atx*-mutated mice displaying the floxed Neo cassette mice with CMV-Cre expressing mice. They were performed on biopsy tails of the pups. The 600-bp band corresponds to the mutated *Atx* Neo excised allele, the 470-bp band corresponds to the *Atx* wild-type allele, while the 3700-bp band corresponds to the mutated *Atx* floxed allele.

65 °C/30 s, 68 °C/4 min) and then 68 °C/7 min, which results in a 470-bp wild-type signal, a 600-bp Neo excised signal and 3700-bp floxed signal; PCR reaction is performed using Long Expand High Fidelity polymerase (Roche®) and reaction buffer 2. Briefly, for Southern blot analysis, genomic DNA was digested with KpnI–SpeI and then hybridized with a 1.0-kb internal probe; *Atx*+/− clones produced an 11.3-kb wild-type signal and 6.1-kb + 5.3-kb targeted Neo-excised signals. F1 male and female heterozygous animals were interbred to obtain *Atx*-mutant mice. Animals were screened by PCR and Southern blot analysis as described above (Fig. 4).

2.4. Mutated autotoxin expression and measure of autotoxin catalytic activity

Standard transfection procedures were used in a further set of experiments. Using Lipofectamin, we transfected several batches of cells. The cells were cultured for a further 24 h. Transfected COS-7 cells were washed twice with phosphate-buffered saline to remove serum and incubated (5 ml for a 10-cm diameter plate; 1 ml for a 3-cm diameter plate) in serum-free DMEM at 37 °C in a humidified atmosphere containing 5% CO_2. The conditioned media were separated from the cells centrifuged to eliminate cell debris, and stored at −20 °C until further use [4]. The fractions were then concentrated and submitted either to SDS–PAGE electrophoresis and Western blot using an anti ATX antibody. Both ATX catalytic activities were measured on this material either the phosphodiesterase or the lysophospholipase D ones, with a colorimetric assay or a thin layer chromatographic assay, respectively, [4]. In brief, the phosphodiesterase activity of ATX was measured by using pNPPP as substrate in test using a modification of the method of Razzell and Khorana (15). Samples (100 µl) were incubated in 96-well plate with *p*-nitrophenyl phenylphosphonate at a 5 mM final concentration in a 50 mM Tris–HCl, pH 9.0 buffer. After 30 min at 37 °C, reactions were stopped by addition of 100 µl of 0.1 M NaOH. The production of *p*-nitrophenol was kinetically quantified by reading the absorbance at 410 nm using a Pherastar plate reader (BMG, Germany) with the appropriate controls. Lysophospholipase D activity was measured by conversion of radiolabeled LPC into radiolabeled LPA. A solution of [^{14}C] palmitoyl-lysophosphatidylcholine (Perkin-Elmer Life Sciences; 55.8 mCi/mmol) at 0.0025 µCi/µL in DMEM supplemented with 1% free fatty acid BSA was first prepared, and 20 µL of this solution was incubated with 500 µl of thawed CM plus 1 µl of sodium orthovanadate (0.5 mM) for 90 min at 37 °C. At the end of the incuba-

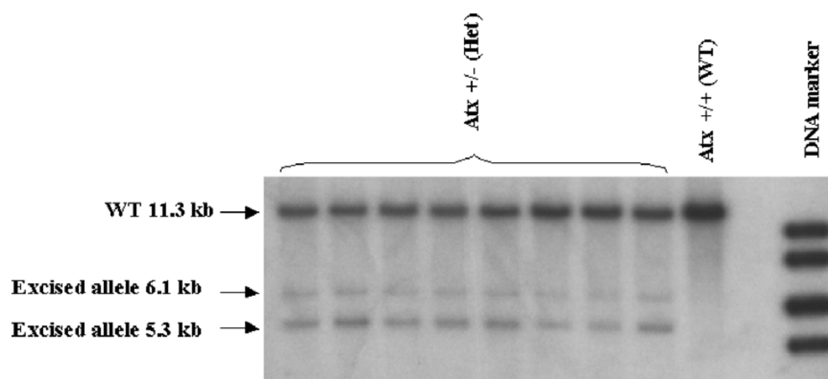

Fig. 4. Southern blot analysis of the offspring of *Atx* floxed mice with CMV-Cre expressing mice. Digestion of tail DNA by KpnI–SpeI resulted in the following diagnostic fragment: wild-type band of 11.3-kb, Neo excised allele bands of 6.1- and 5.3-kb.

tion period, phospholipids were extracted with 500 µl of 1-butanol, evaporated, spotted on a silica gel 60 TLC glass plate (Merck), and separated using $CHCl_3$/MeOH/NH_4OH (60:35:8) as the migration solvent. The plates were autoradio-graphed overnight at −80 °C using a Biomax-MS film (Kodak) to localize radiolabeled LPA spots, which were scraped and counted with 3 ml of scintillation mixture.

3. Results and discussion

L1ES cell clone #3D1-40 bearing the Atx-mutated allele and transfected with CRE expressing plasmid in order to remove the *Lox*P flanked Neo selection cassette, was injected into 121 blastocysts in order to generate Neo-excised Atx knock-in chimeras. It gave rise to the generation of two male chimeras with a 40% chimerism rate. As no agouti mouse was generated from a total of 8 F1 litters, we decided to inject the parental ES cell clones B4, 3D1 and 4G1 harbouring the mutated allele and containing the Neo cassette. This approach was conducted in order to overcome any problem that could have been occurred because of the second round of electroporation of the ES. Three parental ES cell clones (#3B4, 3D1 and 4G1) bearing the mutated Atx allele were injected into 75 blastocysts. Only ES cell clone #3B4 gave rise to seven pups. Three male chimeras, were identified with chimerism rates of 90%, 80% and 60%. These chimeras were then mated with CMV-Cre expressing C57Bl/6 females in order achieve to the Neo cassette deletion in vivo. Breeding of the ES cell clone #3B4 chimeras resulted in the generation of 65 mice from which 40 were agouti (17 males and 23 females). PCR screening revealed they were all wild-type. At that stage, since the size of the litters was not unusual, it seemed that the modification of the ATX locus did not cause any mortality. We hypothesized that the cause of the failure was the slow growing of the ATX mutated ES cells or a potential mixture between the targeted ES cells and wild-type ES cells. More blastocysts were injected with a new lot of ES cells, re-implanted in three gestating females. One out of these three females gave birth again to seven pups. Three male chimera were identified with better chimerism rate than previously, i.e. 85%, 90% and 95%. The three males were mated with three wild-type C57/Bl6 females each. This breeding resulted in the generation of 12 agoutis mice (10 males, 2 females) derived from the 90% chimera. These 12 agoutis F1 mice were screened by Southern blot for heterozygous characterization. Three males and one female were identified therefore suggesting the germline transmission of the mutation and suggesting that the failure in obtaining heterozygous mice from the previous chimeras should be linked to an impaired ability of the ATX-targeted ES cells to colonize the blastocyst and to give to germline transmission. Such germline transmission was unexpected when compared to other KO projects developed concomitantly using the same batch of ES cells. Two of the males were used to produce the F2 generation as above. These males were mated with 2 CMV-Cre-expressing 129v mice each. This resulted in the generation of 47 mice (26 males and 21 females) which were screened for the Cre-mediated excision by PCR and Southern blot. As expected, PCR revealed a band shift from 3.7 kb to 0.6 kb suggesting the Cre-mediated excision of the targeted allele as illustrated on Fig. 4 for six animals. The animals analyzed in lanes 1 and 5 harboured the Cre-mediated excision. At the end of this process, out of 51 pups, 15 heterozygous animals were identified (11 males and 5 females). Two of these heterozygous males

Table 1
Summary of mutated autotaxin homozygous breeding

Mating pairs	Attempt #	Pups	Stillborns	Wild types	Heterozygous	Homozygous
Triad 1	1	6	0	5 (1m + 4f)	1 (1m)	Nil
	2	8	1	2 (1m + 1f)	5 (2m + 3f)	Nil
	3	9	1	3 (1m + 2f)	5 (2m + 3f)	Nil
	4	8	0	5 (2m + 3f)	3 (2m + 1f)	Nil
Triad 2	1	5	0	3 (1m + 2f)	2 (1m + 1f)	Nil
	2	7	0	3 (2m + 1f)	4 (3m + 1f)	Nil
	3	9	0	1 (1f)	8 (4m + 4f)	Nil

Note: each triads were composed of 1 male and 2 females, all heterozygous for autotaxin mutation. The attempts were separated by at least 3 weeks. m: male; f: female.

Table 2
F3 genotypes representation χ^2 analysis

Hypothesis	Theoretical number of animals			Real number of animals			Calculated χ^2 values	Table χ^2 values
	+/+	+/−	−/−	+/+	+/−	−/−		
Viable homozygous	13	25	12	22	28	0	18.59	5.99 (5%) 9.21 (1%)
Embryonic lethal homozygous	17	33	N/A	22	28	0	2.23	3.84 (5%) 6.63 (1%)

were mated with the 4 heterozygous females. These breeding resulted in the generation of seven litters of 5–9 pups each. All of them were tail-genotyped. No homozygous, ATX−/−, animals were born (see Table 1 for details). Considering these results as well as results obtained before, we hypothesized that the ATX deletion was lethal. In order to evaluate statistically this possibility, a χ^2 test was summarized in Table 2.

Such lethality features in deleted mice strain have been scarcely reported in the literature: the main ones were retinaldehyde dehydrogenase-3 [17], the ribosomal protein S19 [18], MAPK [19], Acetyl-CoA carboxylase 1 [20], hexokinase I [21] and a review on attempts around checkpoint-associated proteins, i.e. *ATR*, *chk1*, *Mad2*, *NBS*, *BRCA1* and 2 or *Rad5* [22]. Furthermore, Argraves and Drake reviewed a large panel of lethal situations due to the knock-out of vascular-related genes in mice [23]. They signed the importance of the pathway in which the target gene has been modified or deleted. Concerning ATX, such a result was deceptive but not really surprising, since the catalytic activity of ATX might provide the major source for lyso-phosphatidic acid from lyso-phosphatidylcholine. The cellular responses to LPA are remarkably diverse from cell proliferation and survival to induction of neurite retraction and inhibition of gap functional communication (see Moolenaar [5] and Contos et al. [24] for reviews). Despite this, the targeted, concomitant deletion of one or two LPA receptors has been reported [25,26]. Surprisingly, the $lpa1^{-/-}$ strain presented a series of strong altered traits in their phenotypes, including a 50% neonatal lethality [25], while the double KO $lpa1^{-/-}/lpa2^{-/-}$ strain did not present additional phenotypic traits. The $lpa3^{-/-}$ obtention shaded some light on the possible implication of LPA in embryo implantation [27]. Indeed, such a feature points at a possible key role of LPA into nidification and/or implantation through one of the LPA receptors. On the other hand, the implication of ATX in the blood vessel development was also reported [28] and also might explain the early lack of embryonic implantation and/or development. Conversely, the early death of embryos, or even the lack of development thereof is also an *a posteriori* proof that ATX plays a key role in the production of LPA. Autotaxin might well be the unique source of LPA at least at early stages of the embryonic life. Previous KO attempts of ATX in mice have been very recently reported [29,30]. While there are evidences that ATX is circulating in blood [31] and that LPA production is massively – if not uniquely – by ATX catalytic activity, the role of the ATX polypeptide per se is still not clear. Indeed, initial works have

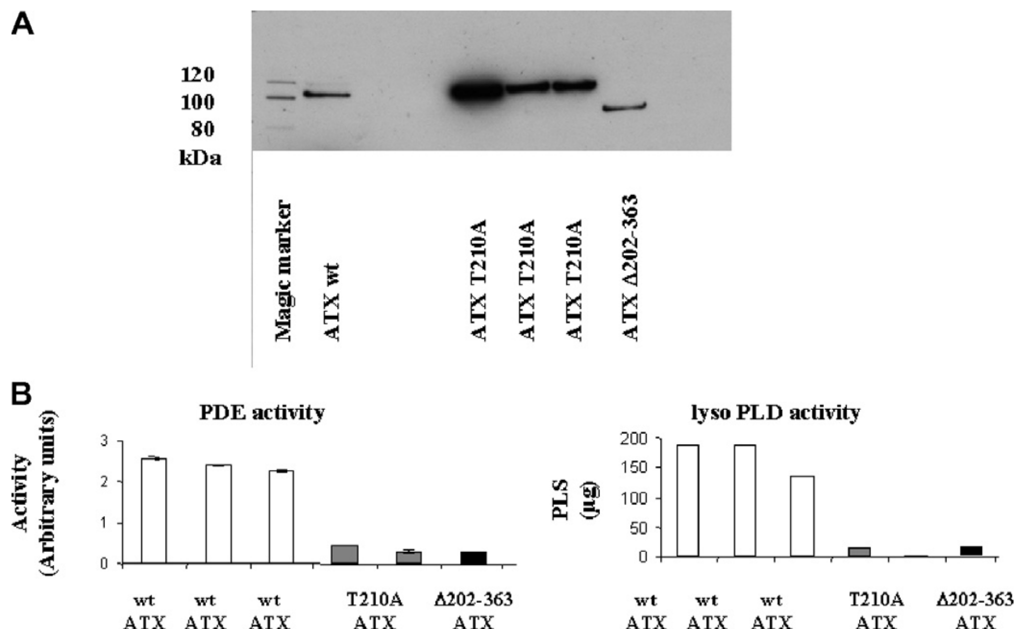

Fig. 5. Expression and catalytic activity of the recombinant autotaxin wild-type and mutants in COS cells. Two mutants (ATX T210A and ATX Δ202–363) were constructed in pcDNA3 vectors. COS cells were transfected and the conditioned media of these cells were checked for the production of the mutated proteins and for their activity, either lysophospholipase D or phosphodiesterase. (A) Western blot analysis of the conditioned media from wild-type (wt), ATX T210A (three lanes) and ATX Δ202–363. (B) Left panel: phosphodiesterase activities using the same preparations as biological sources; right panel: lysophospholipase D activities (open symbols: ATX wild-type -transfected cell-derived conditioned media; shaded symbols: ATX T210A -transfected cell-derived conditioned media and close symbols: ATX Δ202–363-transfected cell-derived conditioned media).

been published according to which ATX might be a motility factor before the lysoPLD catalytic activity was discovered (i.e. before 2002 [2,3]). This fact was at least in part because ATX, a.k.a. NPP2, a nucleotide pyrophosphatase at that time, catalyzed poorly such enzymatic activity and also be cause it bears a RGD sequence, a well-describe motif of association with integrin, although indirect evidence was also brought by the fact that T306A ATX, an catalytic inactive enzyme [15,16], was not able to show any motility capacity.

ATX KO strategy was therefore clearly important in demonstrating or infirming this observation (i.e. if ATX polypeptide was a motility factor by its structure or by its lysoPLD catalytic activity). Tanaka et al. [30] showed that the ablation of the ATX polypeptide was lethal to the embryo. The initiation codon and first 45 amino acids, in their strategy, was replaced by a lacZ sequence, impairing the expression of the whole ATX polypeptide. van Meeteren et al. [29], chose to be more restrictive in their construct, they took off the region of the ATX comprised between exons 5 and 8, a region which encompass the catalytic site of the enzyme. In the present approach, to the contrary of the other reported KO strategies, we decided to maintain the expression of the whole polypeptide, albeit in a catalytic inactive form (the T209A mutant [15,16]).

Despite the fact that the introduction of the mutation in ES cells led to lethality, strongly suggesting that this mutated ATX was indeed the reason for this lethality, the formal proof that the mutated ATX was expressed at early stages of the embryo development was not shown here, while it was reported in the literature that ATX protein is detectable only after 9.5 days in mouse embryos [32]. Indeed, the ES1 cells were screened in RT-PCR for the detection of ATX mRNA. It was still not detectable after 40 cycles, strongly suggesting that ATX mRNA were not present in these cells. Only an indirect demonstration that the ATX mutant was indeed expressed in cells could be done. As reported in Fig. 5, COS cells transfected with either the wild-type or two mutated ATX (T210A and Δ202–363) were shown to be able to express the proteins as detected by our anti-ATX antibody. Nevertheless, no catalytic activity was detectable in the conditioned media of these cells, controlled by identical experiments with the wild-type enzyme, as already reported in the literature [15,16]. These results demonstrate one more time the lethality of the ATX−/− embryos. This demonstrates for the first time that ATX-dependent LPA production is due to the catalytic activity of the enzyme. This is, to our knowledge, the first knockout mice model bearing a single aminoacid mutation that is lethal. It is a wonderful demonstration that the catalytic activity of ATX is key to the normal mouse foetal development. It also strongly suggests that ATX is the major, if not the only – source of lysophosphatidyl acid. Alternative technology will have to be assessed, as conditional knockout after, e.g. tetracycline treatments, or targeted KO in organs (e.g. adipose tissues) after breeding of specific Cre-bearing animals with our strain expression the floxed ATX.

References

[1] Murata, J., Lee, H.Y., Clair, T., Krutzsch, H.C., Arestad, A.A., Sobel, M.E., Liotta, L.A. and Stracke, M.L. (1994) cDNA cloning of the human tumor motility-stimulating protein, autotaxin, reveals a homology with phosphodiesterases. J. Biol. Chem. 269, 30479–30484.

[2] Umezu-Goto, M., Kishi, Y., Taira, A., Hama, K., Domhmae, N., Takio, K., Yamori, T., Mills, G.B., Inoue, K., Aoki, J. and Arai, H. (2002) Autotaxin has lysopholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. J. Cell. Biol. 158, 227–233.

[3] Tokumura, A., Majima, E., Kariya, Y., Tominaga, K., Kogure, K., Yasuda, K. and Fukuzawa, K. (2002) Identification of human plasma lysopholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin a multifunctional phosphodiesterase. J. Biol. Chem. 277, 39436–39442.

[4] Ferry, G., Tellier, E., Try, A., Gres, S., Naime, I., Simon, M.F., Rodriguez, M., Boucher, J., Tack, I., Gesta, S., Chomarat, P., Dieu, M., Maes, P., Galizzi, J.P., Valet, P., Boutin, J.A. and Saulnier-Blache, J.S. (2003) Autotaxin is released from adipocytes, catalyses lysophosphatidic acid synthesis and activates preadipocyte proliferation: up-regulated expression with adipocytes differentiation and obesity. J. Biol. Chem. 278, 18162–18168.

[5] Moolenaar, W.H. (1999) Bioactive lysophospholipids and their G protein-coupled receptors. Exp. Cell Res. 253, 230–238.

[6] Moolenaar, W.H. (2002) Lysophospholipids in the limelight: autotaxin takes central stage. J. Cell. Biol. 158, 197–199.

[7] Gesta, S., Simon, M., Rey, A., Sibrac, D., Girard, A., Lafontan, M., Valet, P. and Saulnier-Blache, J.S. (2002) Secretion of a lysophospholipase D activity by adipocytes: involvement in lysophosphatidic acid synthesis. J. Lipid Res. 43, 904–910.

[8] Boucharaba, A., Serre, C., Gres, S., Saulnier-Blache, J.S., Bordet, J.C., Guglielmi, J., Clezardin, P. and Peyruchaud, O. (2004) Platelet-derivated lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. J. Clin. Invest. 114, 1714–1725.

[9] Jansen, S., Stefan, C., Creemers, J.W.M., Waelkens, E., van Eynde, A., Stalmans, W. and Bollen, M. (2005) Proteolytic maturation and activation of autotaxin (NPP2) a secreted metastasis-enhancing lysophospholipase D. J. Biol. Cell. 118, 3081–3089.

[10] Nam, S.W., Clair, T., Campo, C.K., Lee, H.Y., Liotta, L.A. and Stracke, M.L. (2000) Autotaxin (ATX), a potent tumor motogen, augments invasive and metastatic potential of ras-transformed cells. Oncogene 13, 241–247.

[11] Yang, S.Y., Lee, J., Park, C.G., Kim, S., Hong, S., Chung, H.C., Min, S.K., Han, J.W., Lee, H.W. and Lee, H.Y. (2002) Expression of autotaxin (NPP-2) is closely linked to invasiveness of breast cancer cells. Clin. Exp. Metastas. 19, 603–608.

[12] Boucher, J., Quilliot, D., Praderes, J.P., Simon, M.F., Gres, S., Guigne, C., Prevot, D., Ferry, G., Boutin, J.A., Carpene, C., Valet, P. and Saulnier-Blache, J.S. (2005) Potential involvement of adipocyte insulin resistance in obesity-associated up-regulation of adipocyte lysophospholipase D/autotaxin expression. Diabetologia 48, 569–577.

[13] Ferry, G., Moulharat, N., Fould, B., Rodriguez, M., Giganti, A., Chomarat, P., Galizzi, J.P., Valet, P., Saulnier-Blache, J.S. and Boutin, J.A. (submitted for publication) Autotaxin isoforms: gene organization, tissue distribution, and biochemical characterization and inhibitors, Biochim. Biophys. Acta.

[14] Stracke, M.L., Krutzsch, H.C., Unsworth, E.J., Årestad, A., Cioce, V., Schiffmann, E. and Liotta, L.A. (1992) Identification, purification and partial sequence analysis of autotaxin, a novel motility-stimulating protein. J. Biol. Chem. 267, 2524–2529.

[15] Lee, H.Y., Clair, T., Mulvaney, P.T., Woodhouse, E.C., Aznavoorian, S., Liotta, L.A. and Stracke, M.L. (1996) Stimulation of tumor cell motility linked to phosphodiesterase catalytic site of autotaxin. J. Biol. Chem. 271, 24408–24412.

[16] Koh, E., Clair, T., Woodhouse, E.C., Schiffmann, E., Liotta, L. and Stracke, M. (2003) Sire-directed mutations in the tumor-associated cytokine, autotaxin, eliminate nucleotide phosphodiesterase, lysophospholipase D, and motogenic activities. Cancer Res. 63, 2042–2045.

[17] Dupé, V., Mart, N., Garnier, J.M., Chambon, P., Mark, M. and Ghyselinck, N.B. (2003) A newborn lethal defect due to inactivation of retinaldehyde dehydrogenase type 3 is prevented by maternal retinoic acid treatment. Proc. Nat. Acad. Sci. USA 100, 14036–14041.

[18] Matsson, H., Davey, E.J., Draptchinskaia, N., Hamaguchi, I., Ooka, A., Leveén, P., Forsberg, E., Karlsson, S. and Dahl, N.

(2004) Targeted disruption of the ribosomal protein S19 gene is lethal prior to implantation. Mol. Cell. Biol. 24, 4032–4037.

[19] Xie, Y., Wang, Y., Sun, T., Wang, F., Trostinskaia, A., Puscheck, E. and Rappolee, D.A. (2005) Six post-implantation lethal knockouts of gene for lipophilic MAPK pathway proteins are expressed in preimplantation mouse embryos and trophoblast stem cells. Mol. Reprod. Dev. 71, 1–11.

[20] Abu-Elheiga, L., Matzuk, M.M., Kordari, R., Oh, W., Shaikenov, T., Gu, Z. and Wakil, S.J. (2003) Mutant mice lacking acetyl-CoA carboxylase 1 are embryonically lethal. Proc. Natl. Acad. Sci. USA 102, 12011–12016.

[21] Kanno, H., Murakami, K., Hariyama, Y., Ishikawa, K., Miwa, S. and Fujii, H. (2002) Homozygous intragenic deletion of type I hexokinase gene causes lethal hemolytic anemia of the affected fetus. Blood 100, 1930.

[22] Brown, E.J. (2004) Analysis of cell cycle progression and genomic integrity in ear lethal knockoutsCheckpoint Controls and CancerMethods in Molecular Biology, Vol. 280, pp. 201–212, Humana Press, Totowa, NJ, USA.

[23] Argraves, W.S. and Drake, C.J. (2005) Genes critical to vasculogenesis as defined by systematic analysis of vascular defects in knockout mice. Anat. Rec. A Discov. Mol. Cell Evol. Biol. 286, 875–884.

[24] Contos, J.J., Ishii, I. and Chun, J. (2000) Lysophosphatidic acid receptors. Mol. Pharmacol. 58, 1188–1196.

[25] Contos, J.J., Fukushima, N., Weiner, J.A., Kaushal, D. and Chun, J. (2000) Requirement for the *lpa1* lysophosphatidic acid receptor gene in normal suckling behavior. Proc. Natl. Acad. Sci. USA 97, 13384–13389.

[26] Contos, J.J., Ishii, I., Fukushima, N., Kingsbury, M.A., Ye, X., Kawamura, S., Brown, J.H. and Chun, J. (2002) Characterization of *lpa1(edg4)* and *lpa1/lpa2* (*Edg2/Edg4*) lysophosphatidic acid receptor knockout mice: signaling deficits without obvious phenotypic abnormality attributable to *lpa2*. Mol. Cell. Biol. 22, 6921–6929.

[27] Ye, X., Hama, K., Contos, J.J.A., Anliker, B., Inoue, A., Skinner, M.K., Suzuki, H., Amano, T., Kennedy, G., Arai, H., Aoki, J. and Chun, J. (2005) LPA3-mediated lysophosphatidic acid signaling in implantation and embryo spacing. Nature 435, 104–108.

[28] Nam, S.W., Clair, T., Kim, Y.S., McMarlin, YA., Schiffmann, E., Liotta, L.A. and Stracke, M.L. (2001) Autotaxin (NPP-2), a metastasis-enhancing mitogen, is an angiogenic factor. Cancer Res. 61, 6938–6944.

[29] van Meeteren, L.A., Ruurs, P., Stortelers, C., Bouwman, P., van Rooijen, M.A., Pradère, J.P., Pettit, T.R., Wakelam, M.J.O., Saulnier-Blache, J.S., Mummery, C.L., Moolenar, W.H. and Jonkers, J. (2006) Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development. Mol. Cell. Biol. 26, 5015–5022.

[30] Tanaka, M., Okudaira, S., Kishi, Y., Ohkawa, R., Isei, S., Ota, M., Noji, S., Yatomi, Y., Aoki, J. and Arai, H. (2006) Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysophosphatidic acid. J. Biol. Chem. 281, 25822–25830.

[31] Tanaka, T., Tstustui, H., Hirano, K., Koike, T., Tokumura, A. and Satouchi, K. (2004) Quantitaive analysis of lysophosphatidic acid by time-of-flight spectrometry using a phosphate-capture molecule. J. Lipid Res. 45, 2145–2150.

[32] Bachner, D., Ahrens, M., Betat, N., Schröder, D. and Gross, G. (1999) Developmental expression analysis of murine autotaxin (ATX). Mech. Dev. 84, 121–125.