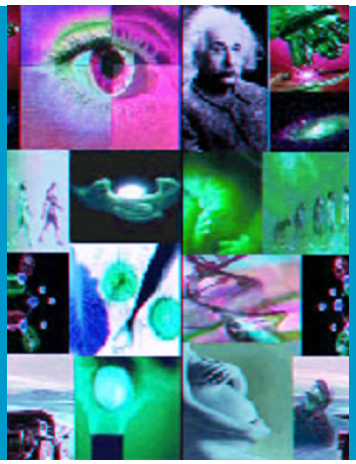
Comisión Nacional de Investigación Científica y Tecnológica - CONICYT



PROGRAMA FONDECYT

INFORME FINAL

ETAPA 2012

COMISIÓN NACIONAL DE INVESTIGACION CIENTÍFICA Y TECNOLÓGICA

VERSION OFICIAL Nº 2

FECHA: 29/08/2014

Nº PROYECTO : 11100408 **DURACIÓN :** 3 años **AÑO ETAPA :** 2012

TÍTULO PROYECTO : KCNN4 AS A MODIFIER GENE OF INTESTINAL CYSTIC FIBROSIS SEVERITY. POSSIBLE ROLE OF MAST CELLS.

DISCIPLINA PRINCIPAL : G2 FISIOPATOLOGIA, FISIOLOGIA CLINICA GE

GRUPO DE ESTUDIO : MEDICINA G2-G3

INVESTIGADOR(A) RESPONSABLE : CARLOS ALEJANDRO FLORES PINILLA

DIRECCIÓN :

COMUNA :

CIUDAD : Valdivia

REGIÓN : XIV REGION

FONDO NACIONAL DE DESARROLLO CIENTIFICO Y TECNOLOGICO (FONDECYT)

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INFORME FINAL
PROYECTO FONDECYT INICIACION

OBJETIVOS

Cumplimiento de los Objetivos planteados en la etapa final, o pendientes de cumplir. Recuerde que en esta sección debe referirse a objetivos desarrollados, NO listar actividades desarrolladas.

Nº	OBJETIVOS	CUMPLIMIENTO	FUNDAMENTO
1	6. To study if mast cells that have mutant CFTR channels present an inflammatory phenotype and if this can be reverted by the inhibition of KCNN4.	TOTAL	We were able to determine that proliferation and migration of mast cells are not affected by CFTR mutations(CftrDF508/DF508). Nevertheless, the genetic or pharmacological silencing of KCNN4 blocked the chemotactic response of mast cells (of wild type and the CftrDF508/DF508 animals) induced by IgE. The proliferation rate was independent of KCNN4 activity in all genotypes. We also determine that CftrDF508/DF508 animals presented increased levels of circulating IgE and TNF-alpha. We reasoned that if IgE was important for mast cells activation in the CftrDF508/DF508 animal the blocking of IgE has to show some improvement on the survival. To test that possibility we generated a CftrDF508/DF508 animal with impaired production of IgE (CftrDF508/DF508/Stat6 -/-). As explained in RESULTS section, the data obtained strongly suggests that IgE depletion improves the survival of the CftrDF508/DF508 mice, indicating that mast cells are key factors in cystic fibrosis intesinal disease.

2	7. To determine if wild type mast cells are migrating into the intestine in response to inflammatory signals released by epithelial and immune cells that are affected by CFTR mutations.	PARCIAL	We tested this possibility by using a mouse model with specific deletion of the Cftr gene from the intestinal epithelium. The use of this animal allows us to test if intestinal epithelial cells are releasing or inducing the release of chemotactic signals for mast cells. The analysis of tissue samples of these animals showed normal number of intestinal mast cells, suggesting that the release of chemotactic signals is triggered by other cell types affected by the CFTR mutation. We also generated a CftrDF508/DF508 mice that did not have mast cells to complete the studies that test the role of these cells in cystic fibrosis intestinal disease. This animal the CftrDF508/DF508/KitW-sh/W-sh, showed improved survival, and taking together this date with those obtained from the CftrDF508/DF508/Stat-/- strongly suggest that mast cell inhibition of absence are improving survival of the CftrDF508/DF508 mice. We need to increase the number of CftrDF508/DF508/KitW-sh/W-sh animals

Otro(s) aspecto(s) que Ud. considere importante(s) en la evaluación del cumplimiento de objetivos planteados en la propuesta original o en las modificaciones autorizadas por los Consejos.

RESULTS OBTAINED:
For each specific goal, describe or summarize the results obtained. Relate each one to work already published and/or manuscripts submitted. In the Annex section include additional information deemed pertinent and relevant to the evaluation process.
The maximum length for this section is 5 pages. (Arial or Verdana, font size 10).

The work plan of the last year of this project involved 2 goals (**6** and **7**). Both goals required the use of animal models to test and expand further the primary observation. We expanded the use of animal models beyond the proposed in the project taking in consideration exiting and unexpected results.

6. To determine if mast cells that have mutant CFTR channels have an inflammatory phenotype and if this can be reverted by the inhibition of KCNN4.

In the first set of experiments (partially anticipated in the previous inform) we were able to isolate and culture mast cells from the animals. We use these cells to test for mast cell functions that could be affected by CFTR mutations. Mast cells are released to the blood stream as undifferentiated precursors from the bone marrow and arrive to target tissues (skin, lungs and intestine, mainly) following chemotactic signals (Halova et al., 2012). Upon arrival these cells start to differentiate, mature and proliferate (Hallgren and Gurish, 2011). We explore 3 ways in which mast cell hyperplasia in the intestine of *Cftr*$^{\Delta F508/\Delta F508}$ mice might be occurring (See Table 1). The first is through a modification in the rate of migration of mast cells. The second an increase in proliferation, and the last by the increase of chemotactic signals responsible for mast cell recruiting. As shown in Fig 1A mast cell proliferation in response to Stem cell factor (SCF), a molecule with known chemotactic properties (Halova et al., 2012). We observed that cells isolated from CF animals are responding equally to SCF those isolated from wild type mice. The absence of KCNN4 in both types of cells is not affecting the rate of proliferation. Fig 1B summarizes the results of the migration assays when cells were stimulated by IgE-DNP crosslinking as the chemotactic agent. Our results demonstrated that CFTR mutations are not affecting mast cell migration when compared to wild type cells. **We also observed that when KCNN4 was absent or inhibited by use of the specific inhibitor TRAM-34 the chemotactic response was completely abolished. CFTR mutations are not affecting proliferation and migration rates in mast cells.**

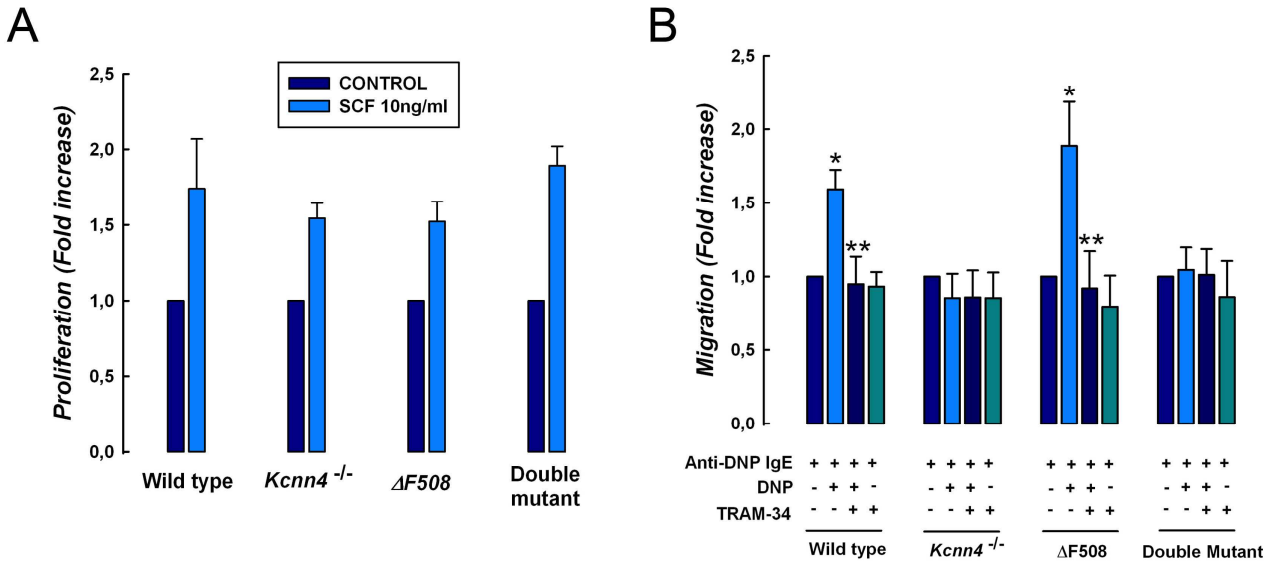

Figura 1. Mast cell proliferation and migration are not affected by CFTR mutations. Graph **A** summarizes mast cell proliferation In the 4 genotypes. No differences were observed when CFTR and/or KCNN4 are genetically silenced (n=6 but double mutant=3). Graph **B** summarizes Transwell migration experiments. * indicates $p<0.05$ compared to respective controls. ** $p<0.05$ compared to DNP stimulus. n=5 for each genotype.

In the attempt to determine if chemotactic agents for mast cells and cytokines are being produced in larger quantities in the *Cftr*$^{\Delta F508/\Delta F508}$ animals we collect intestine and serum samples to test. We measured IgE and IL-4 on intestinal tissues and found no differences in the levels of those molecules in the 4 genotypes. Serum samples were tested for IgE, IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-gamma and TNF-alpha and **we found that both IgE and TNF-alpha presented higher levels** in these samples, nevertheless, the effect of KCNN4 genetic deletion affects the circulating levels of both molecules differentially (Fig 2). While IgE levels are increased when KCNN4 is absent and TNF-alpha is not changed compared to wild type. The IgE results suggested that in in the case of the *Kcnn4*$^{-/-}$ and *Kcnn4*$^{-/-}$*/Cftr* $^{\Delta F508/\Delta F508}$ double mutant mice the higher IgE levels are not harmful as in the *Cftr*$^{\Delta F508/\Delta F508}$ animals because mast cell present in these animals are not properly migrating (Fig 1) and also they release less cytokines (Shumilina et al., 2008). Both impaired functions can reduce mast cell capacity to participate in the inflammatory response and by action of proteases to produce changes on the intestinal mucosa permeability (Groschwitz et al., 2009).

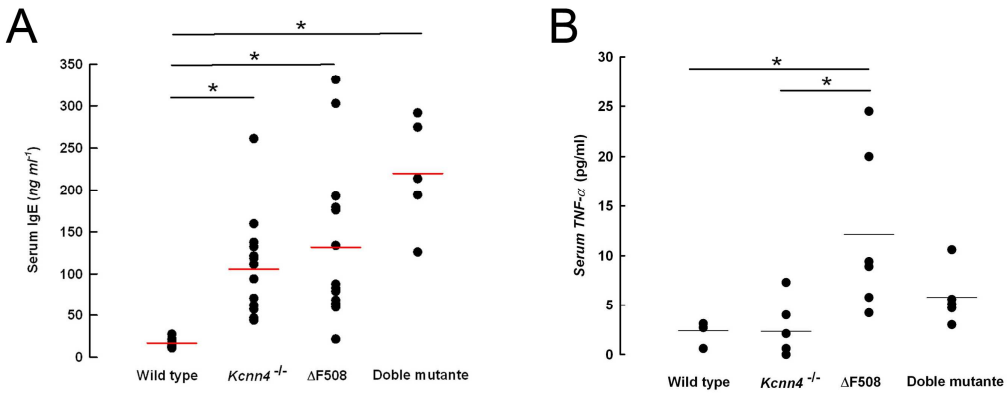

Figure 2. ELISA determination of circulating levels of TNF-alpha and IgE in mice. Graph **A** shows mean (red horizontal lines) and individual measurements (black dots) of IgE on serum. * indicates $p<0.05$ when compared to control. n=10 but double mutant=5. Graph **B** is the data from TNF-alpha measurement showing mean (black horizontal lines) and individual measurements (black dots). * indicates $p<0.05$ compared to wild type. n=5 for each genotype but wild type=3.

Considering that IgE is able to activate mast cells and act as a chemotactic agent as showed in Fig. 1B, we used an animal model that is unable to produce IgE: the *Stat6*$^{-/-}$ mice. We hypothesized that by breeding of a new double mutant animal *Stat6*$^{-/-}$*/Cftr*$^{\Delta F508/\Delta F508}$ we could improve the survival of the animals by inhibiting IgE-dependent mast cell migration and activation. Up to the date we have obtained 5 double mutant *Stat6*$^{-/-}$*/Cftr*$^{\Delta F508/\Delta F508}$ mice and one of them died before 60 days of age by unknown causes. The analysis of tissue samples obtained from the surviving animals demonstrated that the number of mast cells in their intestinal tissues is increased when compared to wild type and *Stat6*$^{-/-}$ mice and this can be due to the existence of other chemotactic signals than IgE (see table 1). Even when the number of animals is too small to draw any statistically significant conclusion about survival this double mutant animals seems to survive better than the *Cftr*$^{\Delta F508/\Delta F508}$ mice. We conclude (preliminary) that the absence of IgE impair mast cell activation, hence their inflammatory activity cannot be triggered.

The meaning of TNF-alpha increased levels on the Cftr animals can be interpreted as a signal of the inflammatory state of CF. This cytokine can be released by several cells of the immune system including mast cells being one of their roles recruit neutrophils (Metz et al., 2007).

7. To determine if wild type mast cells are migrating into the intestine in response to inflammatory signals released by epithelial and immune cells that are affected by CFTR mutation.

To accomplish this Goal we use the *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, a model that lacks mast cells (Grimbaldeston et al., 2005) to breed a double mutant animal *Kit*$^{W\text{-}sh/W\text{-}sh}$/*Cftr*$^{\Delta F508/\Delta F508}$. At the moment we have 4 of these animals that have reached 60 days age and other 2 of 30 days age. This result suggests that when mast cells are not present in the intestinal tissues the survival of the *Cftr*$^{\Delta F508/\Delta F508}$ mice is improved. The second conclusion is that mast cells are key factors in the developing of lethal intestinal disease in mice.

Originally we planned to use this animal to test if signaling from the epithelial cells affected by CFTR mutations were responsible for mast cell recruiting to the intestinal tissue. Nevertheless, this model cannot get rid of cells of the immune system (lymphocytes or ILCs) that could be affected by CFTR mutations and releasing molecules that will ultimately recruit and activate mast cells. Because of that we use a different mouse model: the *Cftr*$^{\Delta 10/\Delta 10}$ animals were generated mating the *Cftr*$^{Flox/Flox}$ with the Villin-Cre mice (Hodges et al., 2008). The resulting animals will bear a specific deletion of the exon 10 of the *Cftr* gene, (that will generate a truncated and non-functional CFTR channel) under the control of the villin promoter that targets the intestinal epithelial cells. Thus this animals lack of CFTR activity in the intestinal epithelium only a feature that we tested by performing Ussing chamber experiments of colon tissue and where all electrogenic chloride secretion is gone. We collected samples of these animals an observed that the number of mast cells in the intestine is similar to that observed in the control mice (see table 1). This result demonstrates that the signals that recruit mast cells in the intestinal tissue of the *Cftr*$^{\Delta F508/\Delta F508}$ animals are not being released by the epithelial cells and also suggest that other CFTR-expressing cells in the intestinal tissue are responsible for mast cell hyperplasia.

Table 1, also compare weight rates for animals used in this study classified by their genotype. As previously shown the increased survival of *Kcnn4*$^{-/-}$/*Cftr* $^{\Delta F508/\Delta F508}$ is not accompanied of weight recovery and the same tendency is observed in the *Stat6*$^{-/-}$/*Cftr* $^{\Delta F508/\Delta F508}$ and *Kit*$^{W\text{-}sh/W\text{-}sh}$/*Cftr* $^{\Delta F508/\Delta F508}$ double mutant mice.

Table 1. Distribution of mast cells in the small intestine and weight of mice.

Genotype	***Small intestine mast cells/field (n)***	***Skin mast cells/field (n)***	***Weight grms (n)***
Wild type	0.35 ± 0.28 (5)	11.2 ± 0.87 (5)	21.4 ± 2 (5)
Kcnn4$^{-/-}$	0.16 ± 0.12 (5)	11.60± 2.80 (5)	24.4 ± 3 (5)
Cftr$^{\Delta F508/\Delta F508}$	1.46 ± 0.40* (5)	12.2 ± 1.20 (5)	13.3 ± 3 (5)*
Kcnn4$^{-/-}$/*Cftr* $^{\Delta F508/\Delta F508}$	0.40 ± 0.19** (5)	12.5 ± 0.57 (5)	13.6 ± 1 (5)*
Stat6$^{-/-}$	0.18 ± 0.09 (4)	13.3 ± 1.56 (4)	n/d
Stat6$^{-/-}$/*Cftr* $^{\Delta F508/\Delta F508}$	0.93 ± 0.09 (4)	14.4 ± 2.05 (4)	12.2 ± 4 (4)
Kit$^{W\text{-}sh/W\text{-}sh}$	0 (4)	0 (4)	n/d
Kit$^{W\text{-}sh/W\text{-}sh}$/*Cftr* $^{\Delta F508/\Delta F508}$	0 (4)	0 (4)	12.0 ± 3 (4)
Cftr$^{\Delta 10/\Delta 10}$	0.13 ± 0.11 (3)	12.8 ± 2.2 (3)	23.0 ± 3 (3)

*indicates $p<0.05$ when compared to Wild type group. ** indicates $p<0.05$ when compared to *Cftr*$^{\Delta F508/\Delta F508}$. Statistical analysis of groups indicated by † will be performed with at least 5 samples.

We need to increase the number of *Stat6*$^{-/-}$/*Cftr* $^{\Delta F508/\Delta F508}$ and *Kit*$^{W\text{-}sh/W\text{-}sh}$/*Cftr* $^{\Delta F508/\Delta F508}$ double mutant mice and *Cftr*$^{\Delta 10/\Delta 10}$ conditional knockouts to perform statistical analysis and survival plots. We also have collected serums of these animals to measure IgE, IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-gamma and TNF-alpha in the samples. Once finished we think that the manuscript will be ready to be sent to publication.

In summary, we have unveiled a IgE-mast cell axis in the *Cftr* $^{\Delta F508/\Delta F508}$ animals. Our data suggest that this is a key mechanism on the occurrence of lethal intestinal obstructions in these animals. By use of animal models we have selectively tackled mast cell function and migration (*Kcnn4*$^{-/-}$/*Cftr* $^{\Delta F508/\Delta F508}$), mast cell activation by deleting IgE expression (*Stat6*$^{-/-}$/*Cftr* $^{\Delta F508/\Delta F508}$) and mast cell presence (*Kit*$^{W\text{-}sh/W\text{-}sh}$/*Cftr* $^{\Delta F508/\Delta F508}$). In all three models the outcome is the same: increased survival when compared to the *Cftr* $^{\Delta F508/\Delta F508}$ animals. The origin of immunological

disturbances on the *Cftr* $^{\Delta F508/\Delta F508}$ mice is not dependent on epithelial malfunction since the *Cftr*$^{\Delta 10/\Delta 10}$ conditional are not presenting mast cell hyperplasia. That important point will remain unknown for the moment.

We also have been working in the role of KCNN4 in the function of neutrophils. We have successfully determined that KCNN4 regulates neutrophil migration in different species including human, horse and mouse. The response of these cells to different chemotactic stimulus is completely impaired when the KCNN4 channels are blocked. We are preparing a second manuscript with these data.

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Shumilina, E., Lam, R. S., Wolbing, F., Matzner, N., Zemtsova, I. M., Sobiesiak, M., Mahmud, H., Sausbier, U., Biedermann, T., Ruth, P. et al. (2008). Blunted IgE-mediated activation of mast cells in mice lacking the Ca2+-activated K+ channel KCa3.1. *J. Immunol.* 180, 8040-8047.

OTHER ACHIEVEMENTS OF THE PROJECT:

- Research visit(s) to other institution(s).
- Outreach activities related to the project's main topic.
- Any other contribution, not addressed elsewhere, that you consider important.

The maximum length for this section is 1 page. (Arial or Verdana, font size 10).

During this project 3 thesis were completed (Texia Riquelme, Pamela Millar and Jose Miguel Arellano), one is about to be finished (Daniel Vera) and 3 started (Jose Ignacio Alvarez, Rodrigo Gonzalez and Amber Philp).

The lab participated in Explora activities helping high school students to set-up small scientific projects and during guided visits to the institute.

The research conducted has been awarded with Best Poster Presentation in the European Cystic Fibrosis Society meeting 2013 (Málaga, Spain).

Our works also were selected for oral presentations in:

Physiological Society meeting (Oxford University 2011).
European Cystic Fibrosis Society (Saint-Máxime, 2012).
15 International Congress in immunology (Milano 2013).

I was invited to give lectures about the lab work in:

Pontificia Universidad Católica de Chile 2011 (Santiago, Chile)
Royal College of Surgeons 2011 (Dublin, Ireland)
Istituto Giannina Gaslini 2011 (Genoa, Italy)
University of Heidelberg 2013 (Heidelberg, Germany)
Universitat de Barcelona 2013(Barcelona, Spain)

The visits to the different labs and institutes have set up (ongoing and future collaborations) with the specialists in the Cystic Fibrosis and Immunology fields.

With Dr Luis Galietta from The Gaslini institute we are working in the developing of new animal models to unveil the role of chloride channels in lung and intestinal epithelium that can be used as new pharmacological targets in cystic fibrosis. We will have one PhD student from Dr Galietta's lab visiting our lab to work in our available animal models.

Marcus Mall from Heidelberg is an expert on cystic fibrosis lung disease. We are planning to work on his animal models to search for immunological aspects of the disease that have been found for us.

PRODUCTOS

ARTÍCULOS

Para trabajos en Prensa/ Aceptados/Enviados adjunte copia de carta de aceptación o de recepción.

Nº : 1
Autor (a)(es/as) : Oliver C, González CA, Alvial G, Flores CA, Rodríguez EM, Bátiz LF.
Nombre Completo de la Revista : Journal of Neuropathology & Experimental Neurology
Título (Idioma original) : Disruption of CDH2/N-Cadherin-Based Adherens Junctions Leads to Apoptosis of Ependymal Cells and Denudation of Brain Ventricular Walls.
Indexación : ISI
ISSN : 0022-3069
Año : 2013
Vol. : 72
Nº : 9
Páginas : 846-860
Estado de la publicación a la fecha : Publicada
Otras Fuentes de financiamiento, si las hay :

Fondecyt 1070241, Fondecyt 1111018, Fondecyt 11090373 and Conicyt BFP.

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http://sial.fondecyt.cl/index.php/investigador/f4_articulos/descarga/12536399/11100408/2012/41765/1/

OTRAS PUBLICACIONES / PRODUCTOS

Sin información ingresada.

CONGRESOS

Nº : 1
Autor (a)(es/as) : Vera, D., Henriquez, C., Riquelme, T.T., Figueroa, C.D., Ehrenfeld, P., Sarmiento, J., Flores.C.A.
Título (Idioma original) : La inhibicion del canal de potasio KCNN4 impide la migracion de neutrofilos
Nombre del Congreso : XXVII reunion anual sociedad chilena de ciencias fisiologicas
País : CHILE
Ciudad : Puerto Varas
Fecha Inicio : 19/11/2012
Fecha Término : 21/11/2012
Nombre Publicación : Biological Research
Año : 2012
Vol. : 45
Nº : A
Páginas : R-55

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http://sial.fondecyt.cl/index.php/investigador/f4_congresos/descarga/12536399/11100408/2012/63086/1/

Nº : 2
Autor (a)(es/as) : Gonzalez, R., Riquelme, T.T., Millar, P., Bustos, V., Cid, L.P., Sepulveda, F.V., Flores, C.A.
Título (Idioma original) : KCNN4 permite el aumento del numero de mastocitos en el intestino de los animales con fibrosis quisqtica por un mecanismo dependiente de IgE
Nombre del Congreso : XXVII reunion anual sociedad chilena de ciencias fisiologicas
País : CHILE
Ciudad : Puerto Varas
Fecha Inicio : 19/11/2012
Fecha Término : 21/11/2012
Nombre Publicación : Biological Research
Año : 2012
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Nº : A
Páginas : R-55
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http://sial.fondecyt.cl/index.php/investigador/f4_congresos/descarga/12536399/11100408/2012/63087/1/

Nº : 3
Autor (a)(es/as) : Vera, D., Henriquez, C., Riquelme, T.T., Figueroa, C.D., Ehrenfeld, P., Sarmiento, J., Flores.C.A.
Título (Idioma original) : Role of KCNN4 Potassium channel in neutrophil chemotactic response
Nombre del Congreso : European cystic fibrosis society. New frontiers in basic science of cystic fibrosis
País : ESPANA
Ciudad : Malaga
Fecha Inicio : 20/03/2013
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Nº : 4
Autor (a)(es/as) : T.T., Riquelme, L.P., Cid, Sepulveda, F.V., Flores, C.A.
Título (Idioma original) : IgE-dependent mast cell hyperplasya in the intestine of a cystic fibrosis mouse model needs KCNN4 channel activity
Nombre del Congreso : 15 international congress of immunology
País : ITALIA
Ciudad : Milano
Fecha Inicio : 22/08/2013
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Nombre Publicación : Frontiers in immunology
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Nº : 5
Autor (a)(es/as) : Riquelme, T.T., Gonzalez, R., Millar, P., Bustos, V., Cid, L.P., Sepulveda, F.V., Flores, C.A.
Título (Idioma original) : KCNN4 inactivation impairs mast cell migration. Role of mast cells in cystic fibrosis intestinal disease.
Nombre del Congreso : Ion Channels in the valley
País : CHILE
Ciudad : Montegrande
Fecha Inicio : 10/04/2013
Fecha Término : 12/04/2013
Nombre Publicación : Proceeding
Año : 2013
Vol. :
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Archivo Asociado :

Nº : 6
Autor (a)(es/as) : Vera, D., Henriquez, C., Riquelme, T.T., Figueroa, C.D., Ehrenfeld, I., Sarmiento, J., Flores.C.A.
Título (Idioma original) : Role of KCNN4 potassium channel in neutrophil chemotactic response
Nombre del Congreso : Ion Channels in the valley

País : CHILE
Ciudad : Montegrande
Fecha Inicio : 10/04/2013
Fecha Término : 12/04/2013
Nombre Publicación : Proceeding
Año : 2013
Vol. :
Nº :
Páginas : 6
Envía documento en papel : si
Archivo Asociado :

TESIS/MEMORIAS

Nº : 1
Título de Tesis : Generacion del animal SPM8-TMEM16A+/+/TMEM16A-/- y el estudio de la distribución del canal TMEM16A en tejidos de ratón.
Nombre y Apellidos del(de la) Alumno(a) : Jose Miguel Arellano Ruiz
Nombre y Apellidos del(de la) Tutor(a) : Carlos A. Flores
Título Grado : Pregrado
Institución : Universidad Austral de Chile
País : CHILE
Ciudad : Valdivia
Estado de Tesis : Terminada
Fecha Inicio : 02/04/2012
Fecha Término : 27/06/2013
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http://sial.fondecyt.cl/index.php/investigador/f4_tesis_memorias/descarga/12536399/11100408/2012/32973/1/

Nº : 2
Título de Tesis : Expresión de Interleuquina 4 mediante método de entrega hidrodinámico de DNA
Nombre y Apellidos del(de la) Alumno(a) : Jose IGnacio Alvarez
Nombre y Apellidos del(de la) Tutor(a) : Carlos A. Flores
Título Grado : Pregrado
Institución : Universidad Austral de Chile
País : CHILE
Ciudad : Valdivia
Estado de Tesis : En Ejecución
Fecha Inicio : 01/06/2012
Fecha Término :
Envía documento en papel : no

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http://sial.fondecyt.cl/index.php/investigador/f4_tesis_memorias/descarga/12536399/11100408/2012/32978/1/

Nº : 3
Título de Tesis : Caracterización de mastocitos STAT6 KO
Nombre y Apellidos del(de la) Alumno(a) : Rodrigo Gonzalez K.
Nombre y Apellidos del(de la) Tutor(a) : Carlos A. Flores
Título Grado : Pregrado
Institución : Universidad Austral de Chile
País : CHILE
Ciudad : Valdivia
Estado de Tesis : En Ejecución
Fecha Inicio : 01/06/2012
Fecha Término :
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GONZALES_R.pdf
http://sial.fondecyt.cl/index.php/investigador/f4_tesis_memorias/descarga/12536399/11100408/2012/32979/1/

Nº : 4
Título de Tesis : Rol de los Mastocitos en la Inflamación Crónica Alérgica de las Vías Aéreas
Nombre y Apellidos del(de la) Alumno(a) : Amber Philp
Nombre y Apellidos del(de la) Tutor(a) : Carlos A. Flores
Título Grado : Pregrado
Institución : Universidad Austral de Chile
País : CHILE
Ciudad : Valdivia
Estado de Tesis : En Ejecución
Fecha Inicio : 01/08/2013
Fecha Término :
Envía documento en papel : no
Archivo Asociado :
Amber_P.pdf
http://sial.fondecyt.cl/index.php/investigador/f4_tesis_memorias/descarga/12536399/11100408/2012/32980/1/
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Nº : 5
Título de Tesis : Regulación de mastocitos en la fibrosis quística por el canal KCa 3.1 (KCNN4)
Nombre y Apellidos del(de la) Alumno(a) : Texia T. Riquelme Mora

Nombre y Apellidos del(de la) Tutor(a) : Carlos A Flores Pinilla

Título Grado : Pregrado

Institución : Universidad Austral de Chile

País : CHILE

Ciudad : Valdivia

Estado de Tesis : Terminada

Fecha Inicio : 21/03/2011

Fecha Término : 29/03/2013

Envía documento en papel : no

Archivo Asociado :

Texia_Riquelme.pdf

http://sial.fondecyt.cl/index.php/investigador/f4_tesis_memorias/descarga/12536399/11100408/2012/33333/1/

Nº : 6

Título de Tesis : Identificación del canal KCNN4 en neutrófilos humanos.

Nombre y Apellidos del(de la) Alumno(a) : Daniel Vera Díaz

Nombre y Apellidos del(de la) Tutor(a) : Carlos A Flores Pinilla

Título Grado : Pregrado

Institución : Universidad Austral de Chile

País : CHILE

Ciudad : Valdivia

Estado de Tesis : En Ejecución

Fecha Inicio : 21/03/2011

Fecha Término :

Envía documento en papel : no

Archivo Asociado :

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http://sial.fondecyt.cl/index.php/investigador/f4_tesis_memorias/descarga/12536399/11100408/2012/33334/1/

Nº : 7

Título de Tesis : Inactivación de la Expresión de Genes Involucrados en Enfermedades Humanas en el Epitelio Intestinal.

Nombre y Apellidos del(de la) Alumno(a) : Pamela Millar Büchner

Nombre y Apellidos del(de la) Tutor(a) : Carlos Flores

Título Grado : Pregrado

Institución : Universidad Austral de Chile

País : CHILE

Ciudad : Valdivia

Estado de Tesis : Terminada

Fecha Inicio : 01/07/2012

Fecha Término : 31/07/2013

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Archivo Asociado :

Resumen_Tesis_Pamela.pdf