ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2018 Випуск 77. С. 76–82
Visnyk of the Lviv University. Series Biology. 2018. Issue 77. P. 76–82

УДК 575.117.5:577.25:615.076.9

NEW NOOTROPIC PREPARATION FROM BLOOD PLASMA (ADEMENT): LACK OF THERAPEUTIC INFLUENCE ON *DROSOPHILA* NEURODEGENERATIVE MODEL

N. Matiytsiv[1], Kh. Dronska[1], O. Makarenko[2]

[1]Ivan Franko National University of Lviv
4, Hrushevskyi St., Lviv 79005, Ukraine
[2]Pereyaslav-Khmelnytsky Hryhoriy Skovoroda State Pedagogical University
30, Suhomlynskyi St., Pereyaslav-Khmelnytsky 08401, Ukraine
e-mail: matiytsiv@yahoo.com

Development and research of new neuroactive agents remain relevant because of neurodegenerative diseases incurability. We tested an experimental preparation created from the peptide blood components of Alzheimer's patients in remission period. It was assumed that this product contained autoneuroactive molecules that might have therapeutic or prophylactic potential. Experimental preparation was tested on *Drosophila* sws-dependent neurodegenerative model. One test system was presented by wild type *Oregon R* and *sws[1]* mutants; another one was presented by transgenic lines for UAS-GAL4 controlled knockdown of *sws* gene in glial cells. Adement and negative control BPAP (blood plasma components of Alzheimer's patients) were fed in two ways: for larvae or on adult stage. The effects of preparations were evaluated by the survival of tested flies. Flies with different genotypes, fed in different ways showed individual survival characteristics in each particular case. However, we did not detect any cases of survival increasing; conversely, in some cases Adement reduced survival. The results showed no influence or toxicity effect of Adement on both control individuals and flies with *sws*-dependent neurodegeneration.

Keywords: neuroactive peptides, Alzheimer disease, *Drosophila*, *swiss cheese* gene, survival test

One of the urgent medical and social problems of the modern world is neurodegenerative diseases, because they are the main reason of disability and untimely death of population in developed countries. According to worldwide statistics, nearly 50 million people suffered Alzheimer's disease (AD) or a related dementia [2]. Neurodegenerative diseases are currently incurable and the number of people who live with dementia is increasing progressively in aging populations. At this time, we have no effective therapy; clinical tests report that few medicines are designed for Alzheimer disease treatment, and 99.6 % of drugs tested turn to be ineffective [9].

Nowadays, one of the promising areas of investigation is the search for potential therapeutic targets for symptomatic treatments of neurodegenerative diseases that may include neuroprotective factors, among these are nootropic drugs and neuroactive peptides [8]. In recent years, peptide neuroprotective agents showed extensive development due to low toxicity, minimal adverse effects, solubility in aqueous media. There is a peptide nature such as Cerebrolysin, which has neuroprotection action on brain [3]. Whereas it is known that invertebrate and vertebrate organisms are able to produce their own neuroactive factors [6, 22], this is the basis for the idea of developing of a medicine with a substance that can be produced in patients with neurodegenerative disease in remission. One of such drugs is experimental preparation Adement that was obtained from the blood of people suffering from Alzheimer's disease in remission [23].

Considering the methodological and ethical issues associated with research on humans, it is recommended to use model objects such as *Drosophila melanogaster.* It is a good test system for primary screening because there are similarities in morphological, biochemical, and functional characteristics between pathological changes in the structure of the brain of neurodegenerative *Drosophila* mutants and humans suffering from neuropathies [10, 16]. Almost all genes related to neurodegeneration in humans have orthologs in the *Drosophila* genome. The large number of stocks with point mutation which characterized neurodegenerative modifications in the brain and possibility to create the controlled knockdown of separate genes in cells of certain types makes *D. melanogaster* a convenient tool for deepening our knowledge of the genetic nature of the pathology of some damages of the CNS and screening of the efficiency of novel experimental remedy. In addition, very important for such research is that *D. melanogaster* has complex brain and nervous system with glial helper cells and that the brain is protected by a barrier akin to the blood-brain barrier.

In recent years, glial cells attract more attention in the study of the mechanisms of neurodegeneration and search for the therapeutic approaches. *Drosophila* has various types of glial cells which play the same role as in the vertebrates: trophic function, regulation of neurotransmitters transport, formation of hemolymph – brain barrier [15, 20]. This is why in our research we used flies with altered function of *swiss cheese (sws)* gene, which is ortholog of human *PNPLA6* gene (Patatin Like Phospholipase Domain Containing 6) also known as a *NTE* (Neuropathy Target Esterase) [18, 26]. *Sws* mutants have glial hyperwrapping around neuron, vacuolization and brain degeneration [14, 24], knockdown of SWS in glial cells leaded to extensive tissue degeneration in corresponding brain part [11, 14]. *Sws* flies are good model of glial-dependent neurodegeneration and testing system for therapeutic agents with possible non-specific influence through glial cells.

In this study, we tested experimental preparation Adement and blood plasma components of Alzheimer's patients (BPAP) as a negative control on *sws*-dependent neurodegeneration *Drosophila* model.

Materials and Methods

Drosophila Stocks. The *sws*^{1} strain was kindly provided by Doris Kretzschmar (USA) [14], and *Oregon-R* provided by the Bloomington Drosophila Stock Center (USA) was used as a control. To create the knockdown of *sws* gene in glial cells, we used Gal4-UAS binary system: the *w*,P{UAS-sws-RNAi}3* strain was obtained from Vienna Drosophila RNAi Center (Austria) and the driver *Repo-Gal4/TM3,Ser* strain was kindly provided by Prof. Karl-Friedrich Fischbach (Germany). In this case, heterozygote driver *Oregon/ Repo-Gal4* was used as a control. All flies were kept in vials in a thermostat under standard conditions (+24 – +25 °C).

Preparations. The subject of study was experimental preparation Adement kindly provided by developers [23]. The method for preparing the agent includes blood sampling, obtaining blood plasma and its aliquoting. The blood sampled in the patient with Alzheimer disease in the course of remission was stored at the temperature of (20±2)°C for (24±2) months or at the temperature (-20±2)°C for (36±6) months. As a negative control, we used similar agent from blood plasma of Alzheimer patients collected on the acute phase of a disease (BPAP).

Treatments and Survival Assays. For treatment, two approaches were used as described before [7]. In one of the examined groups, preparations were added to the standard nutritional medium [4], which was used only for feeding the larvae. Adult flies were kept in this case in preparations-free medium. Larvae feeding based on knowledge about larval stage as main nutritional stage in *Drosophila* ontogenesis [1]. In another group, preparations were fed to flies in the adult stage (imago); preparations were added to the 10 % sucrose solution. Flies were in vials; a filter

paper soaked with the preparations-containing solution was placed at the bottom of the tube. We added blue food color together with Adement and observed blue color of flies' abdomens, thereby confirming the solution was consumed.

The tested preparations were added to the nutritional medium; the potential maximum daily dose for humans (26 μl) was recalculated per 100 ml of the standard nutritional medium or 10 % sucrose solution.

100 flies of each genotype and each examined group were collected, divided into vials of 10 flies, and moved to proper fresh medium every 2–3 days. The number of live flies and those lost to follow-up was counted every 2–3 days. Survival curves were analyzed using Kaplan-Meier plots by GraphPad Prism 6 (Graphpad Software Inc., La Jolla, CA, USA). Log-rank test was used to estimate statistically significant difference.

Results and Discussion

For functional knockout of *sws,* we used panglial driver – Repo-Gal4*,* as described before *UASswsRNAi/RepoGal4* flies demonstrated brain tissue vacuolisation in glia rich area [7]. At larvae feeding, control flies demonstrate definite difference in survival neither with Adement effect nor with BPAP compared with standard medium (Fig. 1, A). Adement led to significant decrease of *UASswsRNAi/RepoGal4* flies lifespan (p≤0.0001), while BPAP had no effect (Fig. 1, B). Data was lower on Adement (p≤0.02) when comparing curves on Adement and BPAP survival

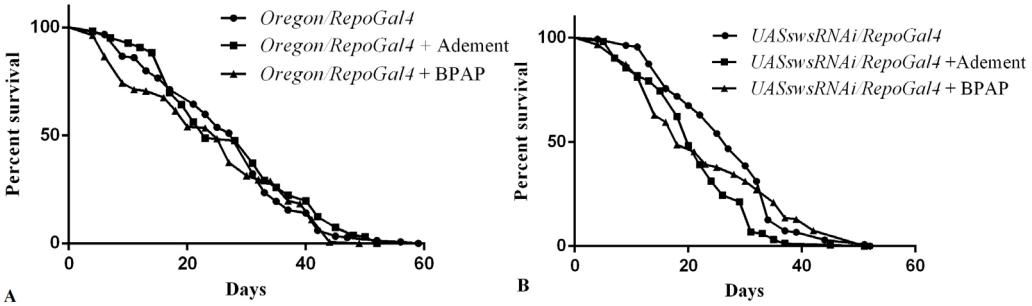

Fig. 1. Survival curves for functional knockout of *sws* in glia *UASswsRNAi/RepoGal4* (B) and control flies *Oregon/RepoGal4* (A) after larva treatment by Adement and BPAP compared to standard medium

At adult feeding, *Oregon/RepoGal4* control flies show significant decrease of survival after Adement and BPAP treatment (p≤0.0001) (Fig. 2, A). Adement and BPAP showed even more negative effect on lifespan of flies with knockdown (Fig. 2, B). However, Adement turned to be more toxic (p≤0.0001) when comparing Adement and BPAP.

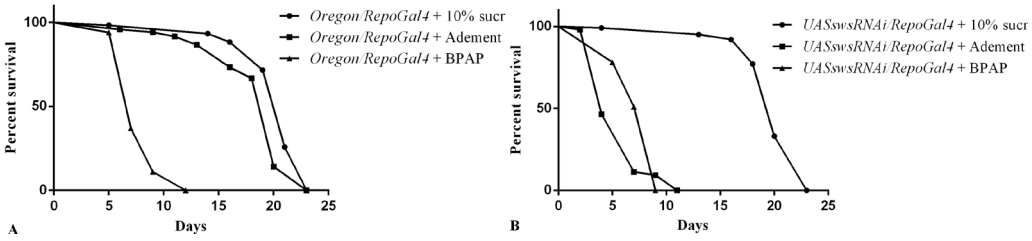

Fig. 2. Survival curves for functional knockout of sws in glia *UASswsRNAi/RepoGal4* (B) and control flies *Oregon/RepoGal4* (A) after adult treatment by Adement and BPAP compared to 10 % sucrose solution

At larvae feeding, Adement did not have any effect on wild type *Oregon R* flies' lifespan, while BPAP substantially shortened it (p≤0.0001) (Fig. 3, A). *sws*^{1} mutants' survival significantly

worsened both after Adement and BPAP ($p \leq 0.0001$); BPAP turned to be more toxic ($p \leq 0.0001$), when comparing Adement and BPAP with each other (Fig. 3, B).

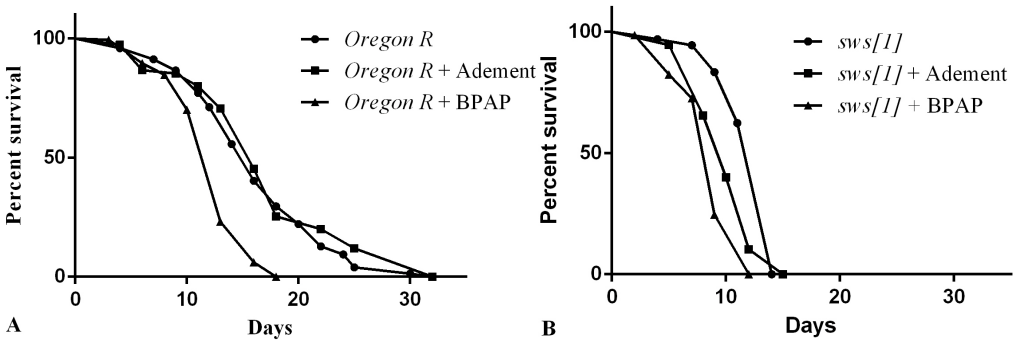

Fig. 3. Survival curves for wild type *Oregon R* (A) and *sws[1]* mutants (B) after larva treatment by Adement and BPAP compeer to standard medium

At adult feeding, wild type *Oregon* lived much worse on both Adement ($p \leq 0.0005$) and BPAP ($p \leq 0.015$) (Fig. 4, A). Both preparations had negative effect on *sws[1]* mutants compared with survival on 10% sucrose, BPAP was more toxic ($p \leq 0.0002$) (Fig. 4, B).

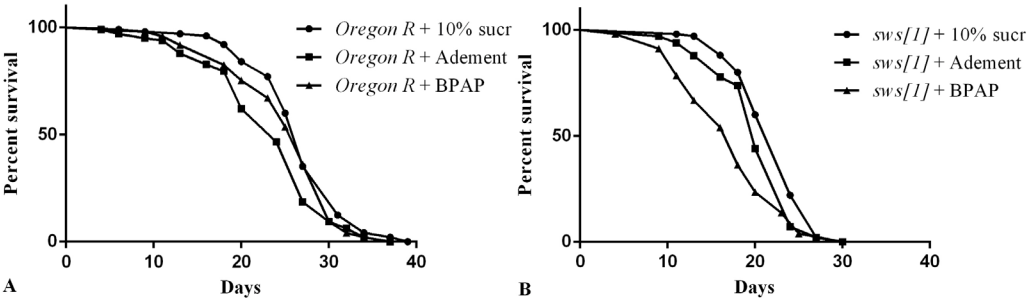

Fig. 4. Survival curves for wild type *Oregon* (A) and *sws[1]* mutants (B) after adult treatment by Adement and BPAP compared to 10 % sucrose solution

In our study, we looked closely at autoplasmtherapy concept of neurodegenerative disorders. The basis of Adement development is the hypothesis about appearance of some curative agents in blood of Alzheimer patients during remission. A possibility of spontaneous temporary remission during AD is still controversial. Usually, improvements are not observed in AD, in contrast, they underline constant progressive degeneration. Nevertheless, there are some evidences about possibility of temporary awakening of a patient with AD [5]. Adement developers assumed appearance of an autocure neuroactive substance in patient's organism, which could accumulate in blood [23]. Following this hypothesis, blood components taken from the same patient in the stage of impairment (BPAP) should be used as a negative control in experimental study in model organisms.

Taking into account that remission is observed not in all Alzheimer's disease patients, Adement preparation can be produced not for each AD patient's autotherapy. However, if effective, it could be used as a general medicine or even serve as a basis for development of prophylactic drug that would solve a problem of prophylactics or delayed induction of disease. Adement is a group of peptides and aminoacids among which small effector molecules or autoneuroprotectors of peptide nature could be found.

There is an assumption that it is protein molecules that have to carry out possible therapeutic function. So, it can be assumed that pathological protein molecules also could be transferred

with patient's blood and cause the disease. One of the warnings of such a procedure was the possibility of transmission of neurodegenerative disorders. However, at this time there is sufficient evidence of the impossibility to get AD through blood transfusion [12, 19].

Drosophila has a complex blood-brain barrier formed by glial cells, this is a highly conservative structure similar to that in the vertebrates. Amino acids and small peptides can transit through the blood-brain barrier in *Drosophila* brain. It is important that almost all molecules transporters in vertebrates have homologs in *Drosophila* [17, 20]. Thus, the human orthologs of SWS - NTE protein, is fully functional when expressed in flies [26]. Moreover, it is known that the expression of mutant human proteins APP, alpha-synuclein, and others in wild-type flies individuals cause the formation of pathological structures and physiological changes [13, 16]. These data prove that Drosophila is an adequate model for human neuroactive peptides study.

In our study, we used two types of fly model systems: a point mutants and transgenic organisms for a targeting gene expression. In such investigation, it is important to combine above mentioned model systems because of differences in genomic and physiological background despite the same gene involving. Another important issue is a way of drug feeding. Different stages of lifespan of vertebrates and invertebrates observed different patterns of gene expression. It is believed that there are small effector molecules which may have stage-specific positive effects in one part of the life span but neutral or negative effects in another part [25] and may have a gene-specific target pattern. In particular, it was shown that experimental peptide preparation Mitochondrin-2 has neuroprotective effect on sws-model flies only in case of larva feeding [7].

The results of our study demonstrated no effect or negative influence of Adement on survival of control and *sws*-dependent model flies. After these results of survival test were obtained, it was of no reasons to do any further research of these drugs on this model. According to our results, Adement did not show therapeutic effect, and in some cases it was more toxic than BPAP, that contradicts initial hypothesis. It is worth stressing that different data obtained for different genotypes, and with influence on different development stages strengthen importance of combining model systems of a single gene, and feeding ways.

We conclude that experimental peptide preparation from blood components of Alzheimer's patients in remission (Adement) had no positive effect on survival of *Drosophila* sws-dependent neurodegeneration model.

REFERENCES

1. *Agrell I., Lundquist A.* Physiological and biochemical changes during insect development; in rockstein M (eds): The Physiology of Insecta. New York: Acad.Press, 1973. Vol. 1. P. 159–233.
2. *Alzheimer's Disease International.* World Alzheimer Report 2016. ADI London, 2016.
3. *Alzheimer's Drug Discovery Foundation*. Cerebrolysin. New York, 2016.
4. *Ashburner M. Drosophila*: a Laboratory Manual. New York: Cold Spring Harbor Lab, 1989. 1331 p.
5. *Bloch F*. A transient awakening of a patient with Alzheimer's disease that questions our practice // Clin. Case Rep. 2016. Vol. 4. N 4. P. 376–378.
6. *Catalani E., De Palma C., Perrotta C., Cervia D.* Current evidence for a role of neuropeptides in the regulation of autophagy // BioMed. Res. Int. 2017. doi: 10.1155/2017/5856071.
7. *Chad M., Artymovych N., Makarenko O., Matiytsiv N.* Effects of mitochondrin-2 on the dynamics of degeneration of brain tissues in *Drosophila* with an altered function of the *swiss cheese* gene // Neurophysiology. 2014. Vol. 6. P. 519–524.
8. *Ciesler J., Sari Yo.* Neurotrophic peptides: potential drugs for treatment of amyotrophic lateral sclerosis and alzheimer's disease // Open J. Neurosci. 2013. Vol. 3. P. 2.

9. *Cummings J., Morstorf T., Zhong K.* Alzheimer's disease drug development pipeline: few candidates, frequent failures // Alzheimers Res. Ther. 2014. Vol. 6. N 4. doi: 10.1186/alzrt269.
10. *Debattisti V., Scorrano L. D. melanogaster*, mitochondria and neurodegeneration: small model organism, big discoveries // Mol. Cell. Neurosci. 2013. Vol. 55. P. 77–86.
11. *Dutta S., Rieche F., Eckl N.* et al. Glial expression of Swiss cheese (SWS), the *Drosophila* orthologue of neuropathy target esterase (NTE), is required for neuronal ensheathment and function // Dis. Model Mech. 2016. Vol. 9. P. 283–394.
12. *Edgren G., Hjalgrim H., Rostgaard K.* et al. Transmission of neurodegenerative disorders through blood transfusion: a cohort study // Ann. Intern. Med. 2016. Vol. 165. P. 316–24.
13. *Kim M., Ho A., Lee J.* Autophagy and human neurodegenerative diseases – a fly's perspective // Int. J. Mol. Sci. 2017. Vol. 18. P. 1596. doi:10.3390/ijms18071596.
14. *Kretzschmar D., Hasan G., Sharma S.* et al. The *swiss cheese* mutant cause glial hyperwrapping and brain degeneration in *Drosophila* // J. Neurosci. 1997. Vol. 17. P. 7425–7432.
15. *Kretzschmar D., Pflugfelder G.* Glia in development, function, and neurodegeneration of the adult insect brain // Brain Res. Bulletin. 2002. Vol. 57. P. 121–131.
16. *Lepesant J.* The promises of neurodegenerative disease modeling // C. R. Biol. 2015. Vol. 338. P. 589–92.
17. *Limmer S., Weiler A., Volkenkoff A.* et al. The *Drosophila* blood-brain barrier: development and function of a glial endothelium // Front Neurosci. 2014. Vol. 8. doi: 10.3389/fnins.2014.00365.
18. *Lush M., Li Y., Read D.* et al. Neuropathy target esterase and a homologous *Drosophila* neurodegeneration-associated mutant protein contain a novel domain conserved from bacteria to man // Biochem. J. 1998. Vol. 332. P. 1–4.
19. *Lyon J.* Study suggests alzheimer and parkinson disease are not transmitted through blood transfusion // JAMA. 2017. Vol. 317. P. 123–124.
20. *Mohylyak I., Chernyk Ya.* Functioning of glia and neurodegeneration in Drosophila melanogaster // Cytology and Genetics. 2017. Vol. 51. P. 202–213.
21. *Mühlig-Versen M., da Cruz A., Tschäpe J.* et al: Loss of *swiss cheese*/neuropathy target esterase activity causes disruption of phosphatidylcholine homeostasis and neuronal and glial death in adult *Drosophila* // J. Neurosci. 2005. Vol. 25. P. 2865–2873.
22. *Quankun H., Binbin W., Jeffrey L.* et al. Circadian rhythm neuropeptides in *Drosophila*: signals for normal circadian function and circadian neurodegenerative disease // Int. J. Mol. Sci. 2017. Vol. 18. doi: 10.3390/ijms18040886.
23. Patent 105240 Ukrainian Patents Database 2016. The method of obtaining a therapeutic and prophylactic preparation "Adement" / Shestunov A., Makarenko O. Published 10.03.2016.
24. *Ryabova E., Matiytsiv N., Trush O.* et al. Swiss cheese, Drosophila ortholog of hereditary spastic paraplegia gene NTE, maintains neuromuscular junction development and microtubule Network; in Perveen F. K. (eds): *Drosophila melanogaster* – Model for Recent Advances in Genetics and Therapeutics. InTech, 2018. doi: 10.5772/intechopen.73077.
25. *Soh J., Marowsky N., Nichols T.* et al. Curcumin is an early-acting stage-specific inducer of extended functional longevity in *Drosophila* // Exp. Gerontol. 2013. Vol. 48. P. 229–239.
26. *Sujkowski A., Rainier S., Fink J., Wessells R.* Delayed induction of human NTE (*PNPLA6*) rescues neurodegeneration and mobility defects of *Drosophila swiss cheese* (*sws*) mutants // PLoS One. 2015. Vol. 10. doi: 10.1371/journal.pone.0145356.

ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2018. Випуск 77

Стаття: надійшла до редакції 18.12.17

доопрацьована 02.03.18

прийнята до друку 13.03.18

НОВИЙ НООТРОПНИЙ ЗАСІБ ІЗ ПЛАЗМИ КРОВІ (ADEMENT): ВІДСУТНІСТЬ ТЕРАПЕВТИЧНОГО ВПЛИВУ НА ЗМОДЕЛЬОВАНУ НЕЙРОДЕГЕНЕРАЦІЮ У *DROSOPHILA*

Н. Матійців[1], Х. Дронська[1], О. Макаренко[2]

[1]Львівський національний університет імені Івана Франка
вул. Грушевського, 4, Львів 79005, Україна
[2]Переяслав-Хмельницький державний педагогічний університет
імені Григорія Сковороди
вул. Сухомлинського, 30, Переяслав-Хмельницький 08401, Україна
e-mail: matiytsiv@yahoo.com

Розробка та дослідження нових нейроактивних речовин залишаються актуальними через невиліковність нейродегенеративних захворювань. Ми перевірили експериментальний засіб, створений із білкових компонентів крові пацієнтів із хворобою Альцгеймера в період ремісії. Передбачалося, що цей засіб містить автонейроактивні молекули, які можуть мати терапевтичний або профілактичний вплив. Експериментальний засіб було перевірено на модельному організмі *Drosophila* зі *sws*-залежною нейродегенерацією. Одну тестову систему було представлено особинами дикого типу *Oregon R* і мутантами *sws[1]*; іншу було представлено трансгенними лініями для UAS-GAL4 керованого нокдауну гена *sws* у гліальних клітинах. Adement і негативний контроль BPAP (білки плазми крові пацієнтів із хворобою Альцгеймера) згодовували двома способами: личинкам або на стадії імаго. Ефекти засобів оцінювали за виживанням досліджуваних особин. Мухи з різними генотипами й оброблені різними способами виявили індивідуальні показники виживання в кожному окремому випадку. Однак ми не встановили жодного із покращенням виживання, навпаки, в деяких випадках Adement знижував виживання. Результати вказують на відсутність ефекту або токсичність засобу Adement як на контрольних особин, так і на мух зі *sws*-залежною нейродегенерацією.

Ключові слова: нейроактивні пептиди, хвороба Альцгеймера, *Drosophila*, ген *swiss cheese*, виживання