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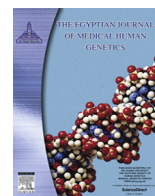


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Review

Autism and *KIR* genes of the human genome: A brief meta-analysisNajmeh Pirzadroozbahani^a, Seyyed Amir Yasin Ahmadi^b, Hamidreza Hekmat^c, Golnaz Atri Roozbahani^d, Farhad Shahsavari^{e,*}^a Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran^b Scientific Society of Evidence-based Knowledge, Research Office for the History of Persian Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran^c Department of Psychology, Islamic Azad University, Borujerd, Iran^d Department of Life Science and Biotechnology, Shahid Beheshti University, Tehran, Iran^e Department of Immunology, Hepatitis Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran

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ABSTRACT

Background: Killer cell immunoglobulin-like receptors (*KIR*) are the transmembrane glycoproteins on natural killer (NK) cells that regulate their functions. Studies show that immune system plays roles in neurodevelopmental disorders like autism, and NK cell abnormality can be a risk factor in autism spectrum disorders.**Aim:** This study aims to investigate the role of *KIR* genes diversity in autism.**Methods:** In order to find the relevant literature, we used PubMed, Google Scholar and other search engines. Association of each gene was analyzed through chi-square with Yate's correction (or Fisher's exact test if necessary). Software comprehensive meta-analysis was used. Both fixed and random effect models were reported.**Results:** Among fourteen genes of *KIR*, the risk role of *KIR2DS1* and *KIR3DS1* were statistically significant based on fixed effect model. Among these two genes, *KIR2DS1* needed random effect model because of its heterogeneity. After applying random effect, its role was not significant. The funnel plot showed no publication bias for *KIR3DS1*, and its role was significant based on fixed effect model ($P = .028$; $OR = 1.31$).**Conclusions:** Autism spectrum disorders are accompanied by *KIR3DS1* which is an activating gene of *KIR*. It seems that hyper-activity of NK cells results in inflammation in neuroimmune system that in turn can be associated with autism. The legend of 3DS1 receptor is unknown, and suggested to be investigated. This meta-analysis should be updated in future.© 2017 Ain Shams University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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1. Introduction

1.1. Rationale

1.1.1. About autism

Autism is a neurodevelopmental disorder specified by verbal and nonverbal communicational and social interaction dysfunctions. The main cause of autism is not clear. Symptoms reveal before the age of 3 and this disorder is more prevalent in boys. Role of economic and social status, life style and the level of education in parents is controversial. Autistic children may show aggression and self-harm behaviors, abnormal responses to people, unusual attachment to objects, resistance to changes, repetitive activities such as flapping, jumping and also hypersensitivity in the five senses [1–3].

1.1.2. About neuroimmunology

Neuroimmune system includes structures and process like biochemical and electrophysiological reactions between nervous and immune systems that protect neurons from pathogens. This system has selective permeable barriers such as brain blood barrier (BBB) and cerebrospinal fluid (CSF) protecting neurons against diseases and also mediating neurons inflammation and trauma. Among all immune cells just mast cells are naturally present in neuroimmune system, because other cells do not pass BBB. When immune responses are needed, they'll be able to pass BBB and be imported in CSF. Neuroimmune system is formed by glial cells initially. For this reason, it is different from peripheral immune system. Immune and nervous systems linked together extensively and intricately by cytokines and neuromediators like neuropeptides. On the other hand immune system function affects many biological processes that we can mention the pathogenesis of neurodegenerative diseases, neurodevelopmental disorders such as autism, and mental health disorders such as schizophrenia. Moreover brain function and development can be affected when innate and adaptive immune responses are not regulated. It's hope that knowing the relation between immune and nervous systems helps us to prevent and treat neurological diseases. Immune system role in autism is a dynamic field of research which can be referred to brain antibodies, serum cytokines, family history and immunogenetics. Proinflammatory cytokines increase like the interleukin (IL)-1beta and interferon (IFN)-gamma produced by peripheral mononuclear cells play role in neurodevelopmental process [4–8].

1.1.3. Natural killer cells in neuroimmunology

Natural killer (NK) cells are a group of immune cells playing role in immune response regulation, and change in their activity is along with immune disorders [9]. These cells are known as the main source cytokines producers, such as IFN-gamma in pathologic and physiologic situations and they are involved in neurodevelopmental disorders like autism. For this reason, some studies have been done about NK cells' role in autism. NK cells abnormality can be a sensitizer factor in autism spectrum disorders, and may prepare the situation for neuroimmune reactions in important developmental periods [10,11]. NK cells have the killer-cell immunoglobulin-like receptors (KIR) interacting with some types of human leukocyte antigens (HLA) [12]. Both KIR and HLA have a variety of genes that each gene is highly polymorphic in turn. KIR has fourteen genes; 8 of them are inhibitory (know with DL in their names) and 6 of them are activating (known with DS in their names). Ten of them are shown in Table 1. These genes are inherited as haplotype pattern and then there are a lot of genotypes in different individuals [13–16]. NK cells have two subtypes; CD56^{dim} and CD56^{bright}. IL-2 induces expression of the CD57 which is observed in matured NK cells and is differentiated form of CD16⁺CD56^{dim} NK cells. These cells have more toxic capacity and are more sensitive to CD16 stimulation, but their response to cytokines and proliferation have decreased. CD57 marker also expresses in CD8⁺ T cells, and is related to close to death phases and cells' old age. In chronic immune activation, CD57 expresses more in T cells [11,17,18].

1.2. Objectives

According to the literature above, we decided to investigate the association of each different genes of *KIR* with risk of autism as a brief meta-analysis; because the place of immunology in behavioral sciences is not clear.

2. Methods and evidence acquisition

Present meta-analysis has been conducted based on Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA, <http://www.prisma-statement.org/>). We used comprehensive meta-analysis 2 software (Biostat, US, <https://www.meta-analysis.com/>).

Table 1
Summary of the collected data from 3 populations.

Gene	Torres et.al. (2012)		Yate's corrected (or Fisher exact) P value (ED)	Guerini. F, et al. (2014) (Italy)		Yate's corrected (or Fisher exact) P value (ED)	Guerini et al. (2014) (Sardinia)		Yate's corrected (or Fisher exact) P value (ED)
	Case N:	Control N:		Case N:	Control N:		Case N:	Control N:	
	158	176		90	250		75	121	
2DL1	153	172	0.7402(-)	89.1	242.5	0.3410(+)	71.25	116.16	1(+)
2DL2	88	96	0.9203(+)	50.4	145	1(-)	42.75	65.34	1(+)
2DL3	144	162	0.9203(-)	77.4	212.5	1(+)	58.5	107.69	0.0557(-)
3DL1	148	174	0.0243(-)	82.8	235	1(-)	72	112.53	1(+)
2DS1	95	67	<0.0001(+)	36	97.5	1(+)	28.5	54.45	0.6021(-)
2DS2	90	97	0.8230(+)	63	140	0.0297(+)	39.75	64.13	1(+)
2DS3	54	46	0.1380(+)	30.6	97	1(-)	21.75	39.93	1(-)
2DS4	154	160	0.0219(+)	81	225	1(+)	72	108.9	0.2865(+)
2DS5	68	53	0.0193(+)	26.1	72.5	1(+)	24	39.93	1(-)
3DS1	83	65	0.0058(+)	36.9	87.5	0.4223(+)	27.75	42.35	1(+)

ED: effect direction. Positive EDs show the genes as a risk factor and negative EDs show the gene as a protecting factor.

2.1. Protocol and registration

The present study has not ever been registered. As well, we searched in PROSPERO (NHS, UK, <https://www.crd.york.ac.uk/PROSPERO/>) in order to find other registered works. The founded records were as following: autism = more than 100; KIR = 1; autism AND KIR = 0.

2.2. Eligibility criteria

All the studies in scientific databases covering both KIR and autism.

2.3. Information sources

We used Google Scholar, Science Direct, PubMed and Web of Science.

2.4. Search

We searched for (KIR OR the complete form) AND (autism OR ASD) in titles.

2.5. Study selection

The process is shown as a flowchart in Fig. 1.

2.6. Data collection process

We were trying to extract data of each KIR genes in both autistic and healthy children. The summary is shown in Table 1.

2.7. Data items

Data of Table 1 are based on absolute frequency of each gene. All percentages were converted to absolute number.

2.8. Risk of bias in individual studies

This risk has been controlled through Yate's correction or Fisher's exact test (each of them with upper P value).

2.9. Summary measures

The forest plots were designed based on two-tailed P values and sample size of each study. The odds ratios were estimated by the software. Since the P values were calculated with Yate's correction or Fisher's exact test, the obtained odds ratios would be underestimated. This protocol has been previously published [19].

2.10. Synthesis of results

The pooled P value and effect size (odds ratio) of each gene were reported based on both fixed and random effect models. No heterogeneity statistical test was used because of low number of imported studies. We used funnel plots instead.

2.11. Risk of bias across studies

It was important to us that all 3 populations should be inside the funnel. In such cases fixed effect model was valid to us, and in genes with bias, random effect model was valid. As well, Yate's correction or Fisher's exact test in turn helped this aim.

2.12. Additional analyses

For family comparison of KIR genes, Bonferroni's correction was adjusted to the pooled P values of the genes.

3. Results

3.1. Studies' characteristics

This meta-analysis covered the information of 323 autistic children and 547 healthy controls From 3 different populations [20,21].

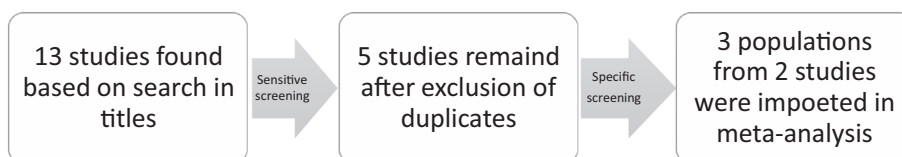


Fig. 1. Document search and screening flowchart.

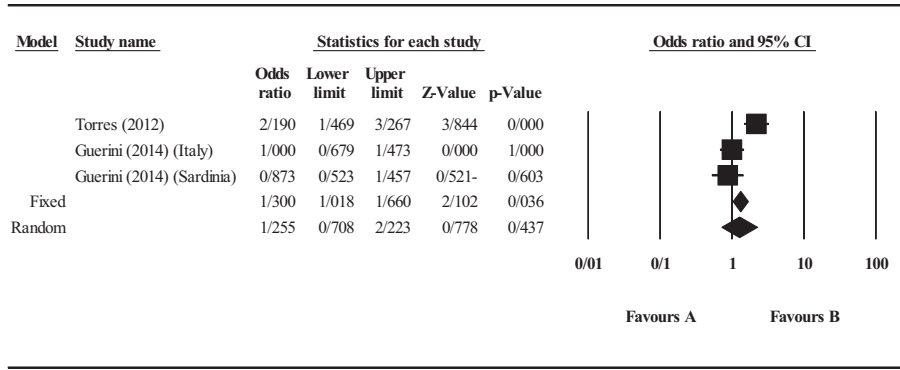


Fig. 2. Forrest plot of KIR2DS1.

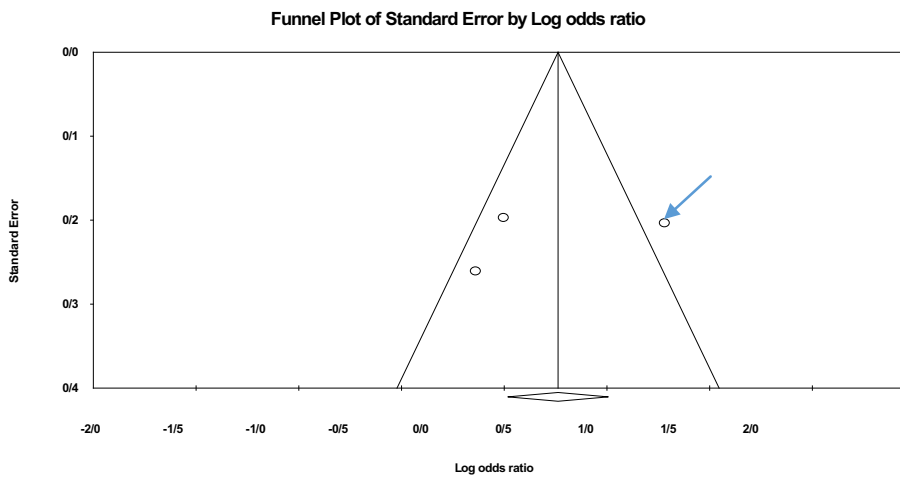


Fig. 3. Funnel plot of KIR2DS1.

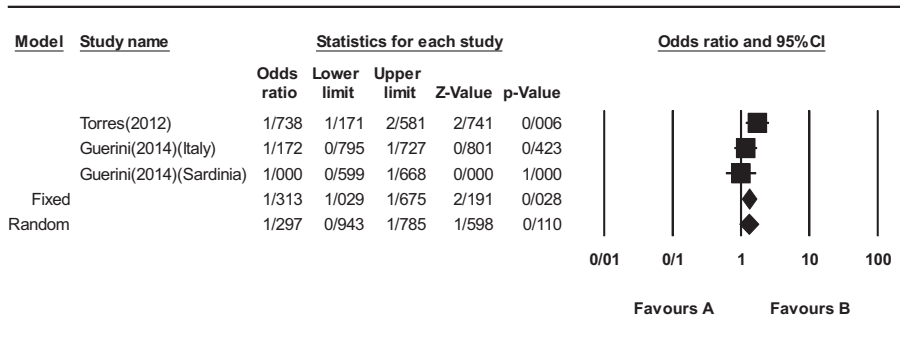


Fig. 4. Forest Plot of KIR3DS1.

3.2. Synthesis and analyses of results

Among the fourteen genes of *KIR*, the risk role of *2DS1* and *3DS1* were statistically significant. No significant relation was found for other genes. Among these two genes, *2DS1* needed random effect model because of its heterogeneity. This heterogeneity was due to the publication bias of Torres et al. study. After applying random effect, it did not remain not significant ($P = .437$) (Figs. 2 and 3). There was no publication bias for *3DS1* and hence the fixed model was valid ($P = .028$; $OR = 1.31$) (Figs. 4 and 5). The meta-graphs of other genes are not shown. Family wise of *KIR* genes, after applying Bonferroni's correction no significant relation would be existed (per family P value > 0.05).

4. Discussion

4.1. Summary of evidence

The first study on this approach, dates back to 2012. Torres et al. as the pioneers of this idea found that autism is associated with *KIR2DS1* especially in combination with its ligand *HLA-C2* [20]. In 2016 they assayed haplotypes of *KIR-HLA* complex in patients [15,16]. Guerini et al. (2014) found that autism was associated with activating *KIR* genes both in children and their mothers. Of course their analyses were without statistical corrections [21]. Gamliel et al. (2016) in a novel work investigated the role of maternal *KIR* and paternal *HLA-C* (because uterine NK cells have maternal

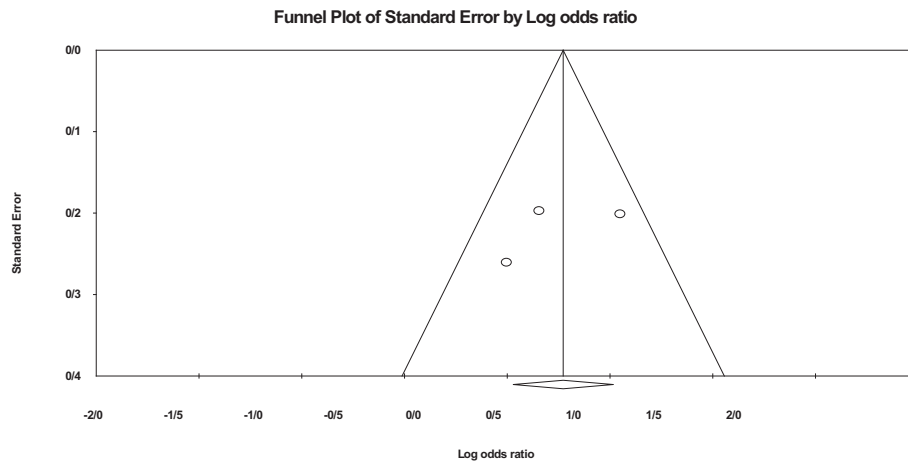


Fig. 5. Funnel plot of KIR3DS1.

source, and half of fetal *HLA-C* has paternal source). In fact they investigated the role of immunology of reproduction in autism. They found that paternal *HLA-C2* accompanied by maternal *KIR2DS1* was associated with autism in children [22]. Of course it is not clear that whether this relation is due to fetomaternal interactions (related to mothers) or due to hereditary (related to children).

4.2. Limitations

Although based on our analyses the association of *KIR3DS1* was more evident than *KIR2DS1*, but there is an important limitation for *KIR3DS1*. The ligand of receptor 2DS1 seems to be *HLA-C2*, but the ligand of protein 3DS1 is still unclear. On the other hand, finding relations for receptor-ligand complex is more evident rather than receptor alone. Therefore researchers were more intended to investigate the role of *KIR2DS1*. Investigation of other ligands like *HLA-G* can also be helpful. Guerini et al. (2017) found that *KIR2DS1 + HLA-C2* complex was more evidently associated with autism in combination with *HLA-G*14* bp insertion [23]. As well, there was a controversy for *KIR2DS1* that its risk relation may not be due to a cause-effect relation, and merely it may be due to a statistical association. Some researchers believe that receptor 2DS1 enables NK cells to fight against autoreactive T cells [18], and because of this, *KIR2DS1* is a protecting factor for multiple sclerosis [13]. Hence it may have a positive role to inhibit pathologic inflammation. Another limitation of ours was that after adjusting family comparison correction, the role of *KIR3DS1* did not remain significant.

5. Conclusion

Autism spectrum disorders are accompanied by *KIR3DS1* which is an activating gene of *KIR*. This relation was based on our meta-analysis, and based on previous works the association was also existed for *KIR2DS1 + HLA-C2* complex. It seems that hyperactivity of NK cells results in inflammation in neuroimmune system that in turn can be associated with autism. The legend of 3DS1 receptor is unknown, and suggested to be investigated. The limitations above should be solved and then this meta-analysis should be updated in future. It should be regarded that *KIR* genes cannot be a diagnostic or prognostic method for autism. Indeed, this work helps us to understand better the physiopathology of autism in order to use in personalized medicine.

Authors' contribution

N Pirzadroozbahani: Search, writing paper and designing forest plots. SAY Ahmadi: Idea, writing paper and statistical consultant. H Hekmat: Psychiatry consultant. G Atri Roozbahani: Genetics consultant. F Shahsavari: Immunology consultant and final approve.

Conflict of interest

None.

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None.

Ethical considerations

Not applicable.

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