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# The Risk of the Next Child Getting Affected by Chronic Granulomatous Disease in Families with at Least One Autosomal Recessive CGD Child

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## ABSTRACT

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disorder more common in autosomal recessive (AR) than X-linked in Iran.

This study aimed to assess whether having a child with AR-CGD would increase the likelihood of the next child being affected by CGD.

Ninety-one families with at least one child affected by AR-CGD entered this study. Out of the 270 children, 128 were affected by AR-CGD. We used a cross tab for the odds ratio (OR) calculation, in which exposure to a previously affected child and the next child's status were evaluated. This study illustrated that the chances of having another child afflicted with AR-CGD are significantly increased if the previous child had AR-CGD (OR=2.77, 95% CI=1.35-5.69).

Although AR disorders affect 25% of each pregnancy, we showed that the chance that the next child would be affected by CGD, given that the previous child was affected, is 2.77 times greater than in families with a normal child. It is recommended to warn families with one or more affected children to evaluate the risk of CGD in their subsequent pregnancies with prenatal diagnosis.

**Keywords:** Autosomal recessive; Chronic granulomatous disease; Parental consanguinity; Siblings

## INTRODUCTION

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disorder with recurrent bacterial and fungal infections. The prevalence of the disease is about 1/200 000 to 1/250 000 individuals worldwide. The approximate estimate of CGD incidence is 1/200 000 in the United States and 1.5/100 000 in Israel.[1,2] CGD is caused by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme deficiency in leukocytes.[3] CGD can be inherited in an X-linked (*CYBB* gene) or autosomal recessive (*AR*) pattern (*CYBA*, *NCF1*, *NCF2*, and *NCF4* genes). Generally, the most common form of CGD is X-linked.[4]

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According to the Mendelian inheritance pattern, the chance of having AR disorders, given that both parents are carriers for the disease, is 25% in each pregnancy. Consanguineous marriages increase the risk of AR and multifactorial disorders.[5] The consanguinity rate varies worldwide, ranging from less than 1% in North America and many European countries to more than 70% in the Middle East, especially in Muslim countries.[6,7] The overall rate of consanguinity in Iran is 37.4%,[8] and it is even higher (65.6%) among families with PID and AR-CGD patients (87.1%).[7]

There are only a few case reports about families with two or more children affected by AR-CGD, and studies on these families are rare.[9-12] This study evaluated the records of children with AR-CGD and the risk of getting affected by AR-CGD in the next sibling of a child with AR-CGD.

## MATERIALS AND METHODS

We assessed the records of 91 Iranian families with at least one child affected by AR-CGD. Patients came from various geographical locations in Iran and had been registered in the Iranian Primary Immunodeficiency Registry (IPIDR) since 2006. These families had a total of 270 children, of whom 128 were affected by AR-CGD; 98 patients were previously.[7]

The diagnosis of CGD was based on clinical manifestations and laboratory tests, including the nitro blue tetrazolium chloride (NBT) test ≤ 10% and dihydrorhodamine 123 assay ≤ 10 in neutrophils after stimulation with phorbol myristate acetate (PMA).[13] Moreover, CGD subtypes were determined by protein immunoblotting. Western blot analysis of the p22, p47, or p67 products was performed in all the cases.[7]

All cases except first children (who do not have a previous child to consider as consecutive) were classified as CGD and non-CGD, as well as consecutive and nonconsecutive. Consecution was considered when the previous child had been affected by CGD. As a result, we had a crosstab that could be used for the odds ratio calculation (Table 1).

The families have stratified into 3 strata: the first cousin, beyond the first cousin, and non-consanguineous marriages. We also assessed the correlation between the number of AR-CGD children and their ratio to all the children in these 3 strata.

Furthermore, the mean number of affected children in these 3 strata was compared using one-way analysis of variance and Tukey's post hoc tests.

**Table 1. Crosstab shows the risk of a child being affected by the chronic granulomatous disease in families with a previously affected child**

| | | Children's Outcome | | |
|---|---|---|---|---|
| | | **Affected child** | **Normal child** | **Total** |
| **Status of the Previous Child** | **Previous Child Affected** | 31 | 14 | 45 |
| | **Previous Child Normal** | 59 | 74 | 133 |
| | **Total** | 90 | 88 | 178 |
| **OR* = 2.77, 95% CI** = 1.35-5.69** | | | | |

*Odds ratio, **Confidence interval

## RESULTS

This study included 91 families with 270 children, of whom 128 had been affected by AR-CGD. 178 children were second or more children among them, 90 had CGD (31 with an affected sibling and 59 with a healthy sibling), and 88 were not affected (14 with an affected sibling and 74 with a healthy sibling) (Table 1). The mean age of the patients was 14.52±8.81 years old, ranging from 1 month to 39 years old. There were 69 males and 59 females. Of the 91 families, 23 had at least two children affected by AR-CGD, with 60 CGD children.

The most remarkable result was the chance of having another AR-CGD in the next child. The odds ratio was calculated according to the crosstab (Table 1), which was 2.77, 95% CI=1.35 to 5.69, showing a significant chance that a child would be affected by CGD if the

previous child had CGD. In other words, the chance for the next child to be affected by CGD in families where the previous child was affected is about 3 times higher than in families with a normal child.

Consanguinity was found in 72 families (79.1%) with 105 AR-CGD children, while non-consanguinity was found in 19 families (20.9%) with 23 AR-CGD children. There was no significant correlation between the number of affected children and consanguinity ($p$=0.335). First-cousin marriages were the most common type of consanguinity in the parents of CGD patients (49 families [52.1%]), compared to marriages beyond first cousins (23 families [25.4%]).

There were 146 children born to 49 families who married first cousins, and 45.89% of those children were affected by AR-CGD. This proportion in families beyond first-cousin marriages was 51.35%. Families with nonconsanguineous marriages had 46% of their children affected by AR-CGD. In summary, there was no significant correlation between the parents' consanguinity (whether first or second cousins) and the total number of children with CGD or successive children getting affected (Table 2).

Of the 23 families with more than 1 child affected by AR-CGD, 19 families had at least 2 siblings with CGD: 1 family with 8, 2 families with 4, 1 family with 3, and the others with 2 consecutive CGD children. Consecutive siblings were seen in 9 of 12 families who had first cousin marriages, 6 of 7 families that had beyond first cousin marriages, and 4 of 4 families with nonconsanguineous marriages. The complete distribution and more details about consecution and consanguinity can be found in Table 1. Of these families, 12 (52.2%) families with 30 AR-CGD children and 7 (30.4%) families with 21 AR-CGD children had first- and beyond first-cousin consanguineous parents. In comparison, 4 (17.4%) families with 8 AR-CGD children had nonconsanguineous parents($p$=0.482).

On Western blot analysis, a defect in p47phox was found in 9 families, p22phox in 6 families, and p67phox in 3 families. Protein immunoblotting was not performed on 5 families because they were out of reach. No significant difference in CGD subtypes was found in the families ($p$=0.223). Also, we find no significant difference in consecutive siblings affected by CGD and CGD subtypes ($p$=0.119).

**Table 2. The number of CGD families is based on the type of marriage and the number of their children**

| Type of Marriage | First Cousin Marriages | Beyond First Cousin Marriage | Nonconsanguineous Marriage | Total |
|---|---|---|---|---|
| **Number of Families, n (%)** | 49 (53.8) | 23 (25.3) | 19 (20.9) | 91 |
| **Number of Patients, n (%)** | 67(52.34) | 38(29.68) | 23(17.96) | 128 |
| **Number of Total Children, n** | 146 | 74 | 50 | 270 |
| **CGD Children/Total Children** | 45.89% | 51.35% | 46% | |

CGD: Chronic granulomatous disease.

## DISCUSSION

There are only a few case reports about families with two or more children with CGD.[7, 9, 10, 12, 14] Our study evaluated 128 AR-CGD patients from 91 Iranian families and assessed the risk of having another child affected by AR-CGD and parental consanguinity in these families.

The relatively high prevalence of AR-CGD in Iran could be attributed to the genetic makeup and high consanguinity rate of the Iranian population;[15] 82.03% of our patients were offspring of consanguineous couples. In comparison, the proportional frequency of consanguineous marriages in Iranians has been reported at 37.4% (8) ($p$<0.05). Our findings are consistent with previously published reports.[7] Nonetheless, we found no significant correlation between the number of affected children and consanguinity. We may conclude that when both parents are carriers, their next child is at a higher risk of being affected by CGD, regardless of consanguinity.

The overall risk of a family having a child with a genetic disorder is 2% to 3%; for first-cousin couples, the risk is about 4% to 7%. The risk can further increase

when there is a family history of a genetic disorder.[16] Although in families with one AR-CGD child where both parents are carriers, the risk of having an affected child should be 25%, we observed a trend in families where about 50% of their children were affected by AR-CGD. Moreover, the chance for the next child to be affected by CGD in families with an affected child is about 3 times higher than in families with a normal child (95% CI=1.35-5.69).

Accordingly, the higher rate of children affected by CGD in these families does not obey the Mendelian rules. Also, it does not relate to how closely related the parents are. It is unknown if any other reason, such as genetic factors, epigenetic modifications, or mere chance, has caused such a rise in these families.

Furthermore, in the families in our study, p47phox deficiency was the most common defect, followed by p22phox and p67phox deficiency, which is in agreement with previous reports.[7,17,18] It shows that the type of AR-CGD in families with more siblings affected by CGD is the same as the overall CGD population. Also, there was no significant difference in AR-CGD subtypes and parental consanguinity.

In conclusion, it is recommended that families with a child affected by CGD be aware of the high risk of CGD in their subsequent pregnancies. Hence, prenatal diagnosis to prevent such occurrences is advised. We also suggest further studies to evaluate the factors influencing the risk of having children affected by AR-CGD in families with consanguineous marriages. Moreover, these results may apply to other inborn immunity errors with the AR trait.

## STATEMENT OF ETHICS

This study was approved in the Ethics Committee of IAARI (IR.TUMS.REC.1399.008).

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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## REFERENCES

1. Chiriaco M, Salfa I, Di Matteo G, Rossi P, Finocchi A. Chronic granulomatous disease: Clinical, molecular, and therapeutic aspects. Pediatr Allergy Immunol. 2016;27(3):242-53.
2. Yu HH, Yang YH, Chiang BL. Chronic Granulomatous Disease: a Comprehensive Review. Clin Rev Allergy Immunol. 2021;61(2):101-13.
3. Yu JE, Azar AE, Chong HJ, Jongco AM, 3rd, Prince BT. Considerations in the Diagnosis of Chronic Granulomatous Disease. J Pediatric Infect Dis Soc. 2018;7(suppl_1):S6-s11.
4. Arnold DE, Heimall JR. A Review of Chronic Granulomatous Disease. Adv Ther. 2017;34(12):2543-57.
5. Bittles AH, Black ML. Evolution in health and medicine Sackler colloquium: Consanguinity, human evolution, and complex diseases. Proc Natl Acad Sci U S A. 2010;107 Suppl 1(Suppl 1):1779-86.
6. Bittles A. Consanguinity and its relevance to clinical genetics. Clin Genet. 2001;60(2):89-98.
7. Fattahi F, Badalzadeh M, Sedighipour L, Movahedi M, Fazlollahi MR, Mansouri SD, et al. Inheritance pattern and clinical aspects of 93 Iranian patients with chronic granulomatous disease. J Clin Immunol. 2011;31(5):792-801.
8. Hosseini-Chavoshi M, Abbasi-Shavazi MJ, Bittles AH. Consanguineous marriage, reproductive behaviour and postnatal mortality in contemporary Iran. Hum Hered. 2014;77(1-4):16-25.
9. Al-Zadjali S, Al-Tamemi S, Elnour I, AlKindi S, Lapoumeroulie C, Al-Maamari S, et al. Clinical and molecular findings of chronic granulomatous disease in Oman: family studies. Clin Genet. 2015;87(2):185-9.
10. Köker MY, Camcıoğlu Y, van Leeuwen K, Kılıç S, Barlan I, Yılmaz M, et al. Clinical, functional, and genetic characterization of chronic granulomatous disease in 89 Turkish patients. J Allergy Clin Immunol. 2013;132(5):1156-63.e5.
11. Köker MY, van Leeuwen K, de Boer M, Celmeli F, Metin A, Ozgür TT, et al. Six different CYBA mutations including three novel mutations in ten families from Turkey, resulting in autosomal recessive chronic

granulomatous disease. Eur J Clin Invest. 2009;39(4):311-9.
12. Prando-Andrade C, Agudelo-Florez P, Lopez JA, Paiva MA, Costa-Carvalho BT, Condino-Neto A. [Autosomal chronic granulomatous disease: case report and mutation analysis of two Brazilian siblings]. J Pediatr (Rio J). 2004;80(5):425-8.
13. Ayatollahi M, Tabei Z, Ramzi M, Kashef S, Haghshenas M. A fast and easy nitroblue tetrazolium method for carrier screening and prenatal detection of chronic granulomatous disease. Arch Iran Med. 2006;9(4):335-8.
14. Elgefors B, Olling S, Peterson H. Chronic granulomatous disease in three siblings. Scand J Infect Dis. 1978;10(1):79-85.
15. Rezaei N, Pourpak Z, Aghamohammadi A, Farhoudi A, Movahedi M, Gharagozlou M, et al. Consanguinity in primary immunodeficiency disorders; the report from Iranian Primary Immunodeficiency Registry. Am J Reprod Immunol. 2006;56(2):145-51.
16. Teeuw ME, Henneman L, Bochdanovits Z, Heutink P, Kuik DJ, Cornel MC, et al. Do consanguineous parents of a child affected by an autosomal recessive disease have more DNA identical-by-descent than similarly-related parents with healthy offspring? Design of a case-control study. BMC Med Genet. 2010;11:113.
17. Rawat A, Vignesh P, Sudhakar M, Sharma M, Suri D, Jindal A, et al. Clinical, Immunological, and Molecular Profile of Chronic Granulomatous Disease: A Multi-Centric Study of 236 Patients From India. Front Immunol. 2021;12:625320.
18. Oikonomopoulou Z, Shulman S, Mets M, Katz B. Chronic Granulomatous Disease: an Updated Experience, with Emphasis on Newly Recognized Features. J Clin Immunol. 2022.