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Identification of Biomarkers for Stunting Through Genetic Variant Analysis

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ABSTRACT

Stunting is a condition of impaired growth and development in children due to chronic nutritional disorders or infections. The risk factor for stunting is predominantly diseases during the 1000 days of life. The incidence of stunting in Indonesia is 21.6%, according to the Indonesian Nutrition Status Survey (SSGI) results. This study aimed to identify stunting biomarkers based on the priority scoring of gene variants. Identification of stunting risk genes used the PubMed based-approach and HaploReg v4.1. We found 33 genes that were identified as stunting risk gene. We then prioritized based on two functional annotation categories: missense-nonsense and cis-expression quantitative trait loci (cis-eQTL). Our analysis found 4 genes as biological stunting risk genes: *MTRR*, *TTF1*, *CASP1*, and *CARD17*. This research demonstrates how genomic variants and bioinformatics approaches can reveal biological insights for stunting.

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1. Introduction

Stunting is a developmental disorder caused by malnutrition, significantly affecting children (Mustakim et al., 2022). This condition's development spans from the fertilization process up to the age of three or four years, heavily influenced by the nutritional status of the mother and child. The World Health Organization (WHO) categorizes stunting as moderate if a child's height is below -2 standard deviations (SD) from the median of the WHO child growth standards, and severe if below -3 SD (World Health Organization, 2018). Stunting initiates in utero and can persist until the child reaches two years of age. A defining characteristic of early childhood stunting is the failure to grow during the critical period of up to the first 1000 days of life, encompassing conception through the second year of life (de Onis & Branca, 2016).

In Asia, the incidence of stunting reach 30-69%, which occurs at a young age, which indicates a failure in child development (Taib & Ismail, 2021). For a decade, stunting has become the focus in Indonesia with an incidence rate of 37% (Beal et al., 2018). According to Sartika et al. in Sambas Regency, Indonesia, the incidence of stunting was high in children aged 9-10 months, namely 20.8% of the 599 children analyzed (Sartika et al., 2021).

Stunting is a health problem among the lower middle class, which is closely linked to the hardships of poverty (Mulyaningsih et al., 2021). In research conducted in Saptosari District, Gunungkidul, Yogyakarta Indonesia exploring the relationship between socio-economic factors and stunting, data revealed that individuals with incomes lower than the UMR (Minimum Regional Wage) were associated with stunting (Somantri, 2022). In the scope of international research, it is also stated that poverty is a risk factor for stunting, with an odds ratio (OR) of 1.7 ; (95% CI,1.6-1.8; P < .001).

The chance of stunting in children is caused by various things, for example, eating habits, history of infection, family economic status, parents' education, environ mental cleanliness, and hygiene (Natale & Rajagopalan, 2014). Other risk factors are the baby's birth length, the mother's lack of education, and the mother's low body mass index (BMI) (Li et al., 2020). In addition, it is also stated that stunting is affected by the interaction of genetic entities (Natale & Rajagopalan, 2014). According to Stewart et al., heredity plays an important role, with a percentage of 80%, and the remaining portion is caused by hormones, malnutrition, and economic and environmental factors (Taib & Ismail, 2021).

Genetic studies, drawing from the GWAS database, have identified 1033 single nucleotide polymorphisms (SNPs) associated with short family stature in Taiwan, among which 13 SNPs were found to be susceptible to stunting, identified as candidate SNPs for stunting susceptibility (Lin et al., 2017). Another study in China identified a gene associated with short stature that affects stunting, namely *PTPN11* (Inoue et al., 2011). This genomics study has the potential to develop improved treatments by targeting the pathophysiology underlying stunting. However, in clinical practice, genomics research is seldom conducted to evaluate the determination of appropriate stunting therapy, especially in Indonesia.

2. Materials and Methods

In Fig. 1 shows an integrative analysis of database utilization for identifying stunting biomarkers. Identification of SNPs were extracted from PubMed and futher analysis by HaploReg v4.1 with the criteria $r^2 > 0.8$.

2.1. Literature search using PubMed

Identification of genes at risk of stunting was carried out by systematically searching the PubMed database for articles related to stunting during 2013-2021 period. Keywords used in the search include "stunting", "short stature", "polymorphism", "SNPs", "growth". The criteria used in the search were: 1) testing for human sample; 2) stunting clinical phenotype; 3) SNPs reported be associated with stunting

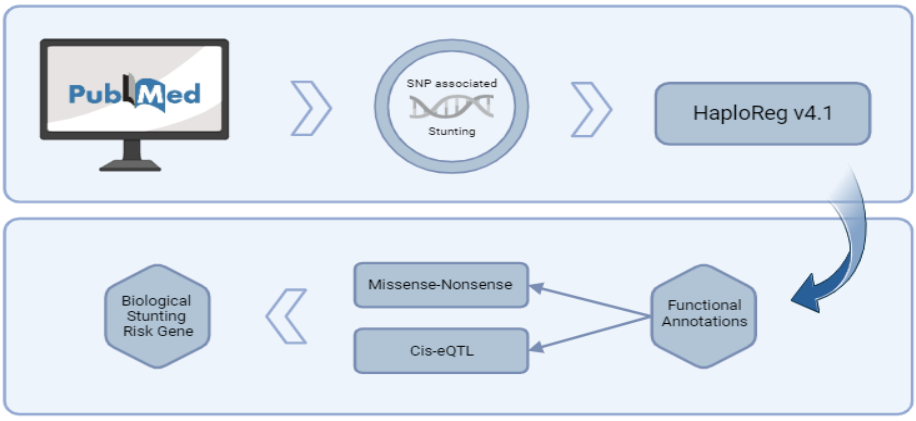

Fig 1. Schematic model demontrates the biomarkers for stunting

2.2. Biological stunting risk gene

The biological stunting risk genes were obtained from annotation functional categories that met two or more criteria (i.e., had a score of 2). First, missense-nonsense mutation based on HaploReg v4.1, with criteria $r^2 > 0,8$. Then, we consider the cis expression quantitative trait loci (cis-eQTL) to protein expression in small intestine, whole blood and thyroid tissue. cis-eQTL explain gene expression in tissues that related to stunting. Each annotation is given a score of 1. Each annotation is given a score of 1. Gene with score ≥ 2 referred as biological stunting risk gene.

3. Results and Discussion

3.1. SNPs Associated with Stunting

In developing this study, we used PubMed to identify potential genes as candidate biomarkers for stunting. The parameters we use to investigate candidate genes as stunting biomarkers use two annotation categories, namely missense-nonsense mutations and cis-eQTL. The discovery of these biomarkers can be used to diagnose disease quickly and accurately (Lopez, 2018). From the bioinformatic pipeline, we found 56 SNPs associated with risk of stunting (Table 1).

Table 1. SNPs Associated stunting genes were identified through pudmed and candidate gene studies

SNPs Associated stunting			
rs9925287	rs3771381	rs7690457	rs509035
rs472177	rs10935120	rs6831357	rs11568820
rs11801053	rs7632381	rs10166966	rs4516035
rs572248	rs13131350	rs10252253	rs11568820
rs3135955	rs6845999	rs556386	rs4516035
rs61736596	rs4240326	rs1174658	rs8178087
rs7629425	rs6470764	rs542571	rs9840993
rs3796164	rs12338076	rs2794385	rs438363
rs1143681	rs4842838	rs437334	rs6519902
rs2037089	rs258324	rs369720	rs9925287
rs1254392	rs4308051	rs12927001	rs11801053
rs10177957	rs10380	rs1174657	rs61736596
rs73403546	rs292215	rs12021720	rs472177
rs2665802	rs3791679	rs572248	rs9226

3.2. Biological Stunting Risk Gene

The obtained SNPs were further identify using HaploReg v4.1 with the criterion $r^2 > 0.8$. Our identification showed 33 genes associated with stunting. Biological stunting risk genes were analyzed using two biological functional annotation criteria. Each gene that assigned criteria is given score 1. We found missense/nonsense annotation risk of stunting (n = 21) genes; genes with cis-eQTL (n=16). Then, there is 4 genes with scores that met the criteria (Table 2).

We used two criteria annotation to evaluate each gene in the stunting risk. First annotation, we considered missense-nonsense mutation. Missense-nonsense mutation occur by substituting an amino acid in a protein that changes the gene code. Then, second annotation we considered cis-eQTL to prioritize the stunting associated gene. This annotation provides an overview of how the variant affects protein expression in the tissue involved. Fig. 2 showed connection between the gene-associated stunting and the scoring system using functional annotation.

Two functional annotation criteria were used to determine the biological stunting risk gene. Each gene gets a score based on the number of measures it fulfills (scores from 0 to 2 for each gene). Assessment of each of the 33 candidate genes with the following criteria: (1) nonsense risk variant (n

= 21); (2) genes with cis-eQTL effects (n = 16). There are 4 genes with a total score ≥ 2 and categorized as a ‘ biological stunting risk gene’ (Table 2) (Fig. 2). The stunting risk genes include *MTRR, TTF1, CASP1,* and *CARD17.*

Table 2. Gene associated with s tunting were proiritized using functional annotation

GENCODE ID	GENCODE name	missense-nonsense score	cis-eQTL score	total s core
ENSG00000124275	*MTRR*	1	1	2
ENSG00000125482	*TTF1*	1	1	2
ENSG00000137752	*CASP1*	1	1	2
ENSG00000255221	*CARD17*	1	1	2
ENSG00000065534	*MYLK*	1	0	1
ENSG00000069122	*GPR116*	1	0	1
ENSG00000117983	*MUC5B*	1	0	1
ENSG00000130589	*RP4-697K14.7*	1	0	1
ENSG00000137992	*DBT*	1	0	1
ENSG00000147697	*GSDMC*	1	0	1
ENSG00000156218	*ADAMTSL3*	1	0	1
ENSG00000163257	*DCAF16*	1	0	1
ENSG00000168907	*PLA2G4F*	1	0	1
ENSG00000172289	*OR10V1*	1	0	1
ENSG00000173627	*APOBEC4*	1	0	1
ENSG00000188155	*KRTAP10-6*	1	0	1
ENSG00000204414	*CSHL1*	1	0	1
ENSG00000221989	*OR2A2*	1	0	1
ENSG00000225614	*ZNF469*	1	0	1
ENSG00000233611	*AC079135.1*	1	0	1
ENSG00000255177	*RP11-532E4.2*	1	0	1
ENSG00000007312	*CD79B*	0	1	1
ENSG00000105499	*PLA2G4C*	0	1	1
ENSG00000129055	*ANAPC13*	0	1	1
ENSG00000137757	*CASP5*	0	1	1
ENSG00000143344	*RGL1*	0	1	1
ENSG00000162704	*ARPC5*	0	1	1
ENSG00000166900	*STX3*	0	1	1
ENSG00000177311	*ZBTB38*	0	1	1
ENSG00000182923	*CEP63*	0	1	1
ENSG00000185324	*CDK10*	0	1	1
ENSG00000251168	*CTD-2072I24.1*	0	1	1
ENSG00000254477	*AP000640.10*	0	1	1

Methionine synthase reductase (*MTRR*) has an essential role in homocysteine metabolism. *MTRR* synthase will be distruped due to low levels of vitamin B12 and folic acid (Barbosa et al., 2008). Vitamin B12 is necessary for average growth and physiological function in synthesizing neurotransmitters, synaptogenesis, and nerve myelination. Vitamin B12 deficiency will result in nerve damage or brain atrophy (Sourander et al., 2023). In addition, a shortage of vitamin B12 during pregnancy can cause abortion, low birth weight, and premature birth (Finkelstein et al., 2019) — meanwhile, folic acid functions in the growth and development of the fetus. Folic acid deficiency

during pregnancy will cause anemia in pregnant women and congenital abnormalities in the fetus (Greenberg et al., 2011).

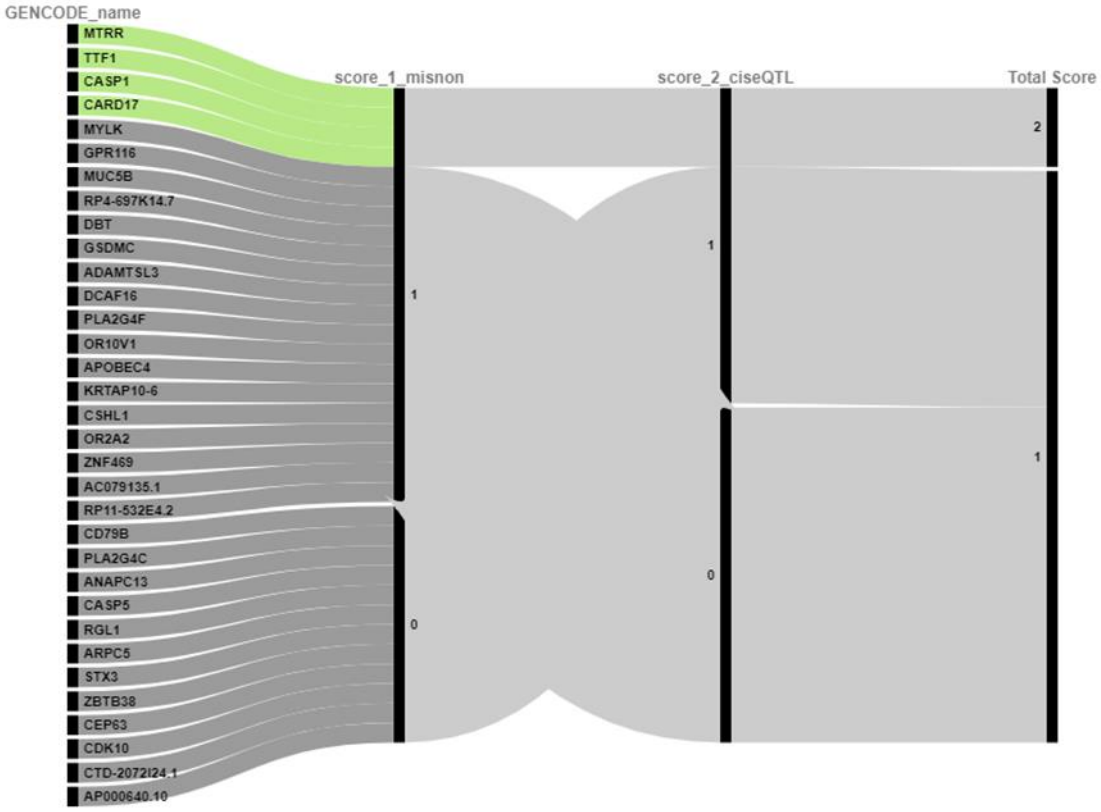

Fig 2. Connection between the gene-associated Stunting and the scoring system using functional annotation

Thyroid Transcription Factor 1 (*TTF1*), commonly known as NK2 homeobox 1 (NKX2-1) is a gene expressed in the thyroid gland, lungs, and fetal brain during embryonic development (Chen et al., 2018). This gene functions in the formation of the thyroid organ and produces hormon (Berto-Júnior et al., 2018) and promotes lung maturation and morphogenesis (Phelps et al., 2018). During lung development, *TTF1* expression will increase in respiratory epithelial cell in the conductive and peripheral airways during lung formation (Chen et al., 2018; Pohlenz et al., 2002). Mutation in *TTF1* gene cause respiratory disease and growth restriction (Berto-Júnior et al., 2018).

One of the main components in the inflammasome is Caspase-1 (*CASP1*) (Lappas, 2014). *CASP1* must be activated through the assembly of the inflammatory complex to initiate its activity. The production and storage processes are in the network as zymogen (Molla et al., 2020). Interleukin (IL)-1β is the target of the *CASP1* molecule, which is the cause of premature birth due to intrauterine infection or inflammation the highest expression of this protein in the myometrium and fetal membranes (Lappas, 2014). *CARD17* inhibits IL-1-dependent *CASP1* expression, where this gene will hinder the formation of CASP1. According to Kleinbrink et al *CARD17* plays a role in inhibiting filament assembly and *CASP1*, which is mediated by the caspase recruitment domain (ASC) (Kleinbrink et al., 2021). However, the exact mechanism by which *CARD17* is linked to stunting is unknown. Our study has limitations that require careful consideration. Further studies are needed to confirm the results obtained.

4. Conclusion

In summary, our bioinformatics study suggests potential biomarkers for stunting such as *MTRR*, *TTF1*, *CASP1*, and *CARD17*. However, further research is required to confirm the relevance of thede genes' regulation as clinical biomarkers and to identiry thepeutic targtets.

Author Contributions: Anisa Devi Kharisma Wibowo, Anisa Nova Puspitaningrum, Lalu Muhammad Irham designed the study and performed data analyses. Anisa Devi Kharisma Wibowo, Anisa Nova Puspitaningrum interpreted the results. Anisa Devi Kharisma Wibowo, Anisa Nova Puspitaningrum, Muhammad Ma'ruf, Lalu Muhammad Irham, Woro Supadmi, Ayu Lifia Nur Kartikasari, Wirawan Adikusuma, and Rockie Chong, Firman Firman, Media Fitri Isma Nugraha, Lalu Muhammad Harmain Siswanto, Sabiah Khairi, Satria Pranata reviewed, revised, and edited the manuscript. Anisa Devi Kharisma Wibowo wrote the manuscript. Lalu Muhammad Irham supervised the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare no conflict of interest.

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