SYSTEMATIC / NARRATIVE REVIEW ARTICLE

Identifying an appropriate gene testing tool for inherited retinal dystrophy in Indonesia, a developing country

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ABSTRACT

Introduction: Inherited retinal dystrophy (IRD) is a group of untreated genetic ocular diseases that mostly affect young people. The number of patients with IRD worldwide, including in developing countries, is growing each year. This literature review aimed to investigate the current utilised genetic screening of IRD worldwide and to propose the most feasible genetics test and diagnostic method for IRD in developing countries, especially Indonesia.

Materials and Methods: A literature search was performed in PubMed and Google Scholar databases. Papers conducting wide genome sequencing, including panel sequencing (panel-seq), microarray, whole exome sequencing (WES), whole genome sequencing (WGS) and Sanger sequencing on patients with IRD, were included. Papers were sorted into several groups to visualise the sequencing technology's detection rate. Detection rate comparison analysis was done using the meta-regress protocol in the R program. Whereas the number of novel mutations in each testing tool each year was pooled and compared in the graph.

Results: After conducting the literature study, 37 papers were sorted from 451 results. Most studies conducted a panel-seq with 16 records followed by WES with seven records. The detection rate of the WES meta-analysis was 0.66, which was slightly better than the panel-seq with 0.55. The number of novel mutation discoveries fluctuated each year with panel-seq as the most prominent finder. Cost factors and the limitation of sequencing devices make panel-seq a more appropriate tool in Indonesia.

Conclusion: The most effective selection for evaluated genetic testing was WES. Therefore, panel-seq is more suitable for first-tier genetic testing in Indonesia.

KEYWORDS:
Inherited retinal dystrophy, whole genome sequencing, diagnostic rate, novel mutations discovery, suitable genetic testing

INTRODUCTION

Inherited retinal dystrophy (IRD) is a group of untreated genetic ocular diseases that mostly affect young people.[1] Disease manifestations and genetic background of IRD are heterogeneous. So far, 281 genes have been associated with IRD (https://web.sph.uth.edu/RetNet/home.htm). The damaged retinal cells tend to compromise the patient's sight partially or completely. At the minimum, 20 IRD types were identified including retinitis pigmentosa (RP), Stargardt disease, rod-cone dystrophy (RCD) and Leber congenital amaurosis (LCA).[2-4]

The number of people around the world affected by IRD is increasing every year, and approximately one in 3,000 to 5,000 individuals are affected by IRD.[5] However, the disease prevalence in Indonesia was not available until this paper was finished. The actual estimation is challenging since the advanced diagnostic tools are not evenly distributed across all nations. Furthermore, suspected IRD patients must be diagnosed by a vitreo-retina specialist.

IRD is also known to severely decrease young people's quality of life and poses a heavy psychological and economic burden. People's awareness of getting a check-up for any IRD symptoms varied in the global population.

The clinical features of IRD vary among individuals, but the key features of each type of IRD are unique and often include retinitis pigmentosa with arteriolar attenuation, retinal pigmentary changes (hypopigmentation/hyperpigmentation of bone-spicule and pigment clumping) and waxy disc pallor. Several IRDs exhibit similar features at the late stages, such as severe retinal cell death, extensive atrophy of the retina, and irreversible visual loss.

To further clarify the diagnosis, these genetically heterogeneous retinal dystrophies, such as cone dystrophies (CD), cone-rod dystrophy (CRD), Leber congenital amaurosis, and retinitis pigmentosa, present significant challenges, since mutations can be expected in any of 8–61 genes. A powerful screening method for these genes or variants with cost-effectiveness was needed.

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Commonly used genetic testing tools globally in IRD cases include Sanger sequencing, microarray and next generation sequencing (NGS) technology. The NGS technology can be costumed into panel-based NGS (panel sequencing [panel-seq]), whole exome sequencing (WES), and whole genome sequencing (WGS). These technologies' principles were different and yielded varied diagnostic rates.

Although all sequencing instruments (Sanger and NGS) were available in Indonesia, genetic screening of IRD was uncommon in Indonesia. Genetic screening was limited to the field of research.[6,7] The genetic screening is not included in the diagnosis due to high prices and the clinical meaning. Currently, it is not feasible, and the genetic data is not used for further treatment consideration.

Ideally, patients with known genetic backgrounds can be treated more effectively than those with unknown ones. To begin a big plan for genetic therapy trials in Indonesia, the Indonesian team must begin to classify their patients.

In Indonesia, people with no or light symptoms are not likely to visit an ophthalmologist, while those suspected with severe symptoms are less motivated to be referred to the tertiary hospital after knowing that their condition is untreated. Similarly, the lack of awareness couple with IRD symptoms could produce an affected baby since the accumulation or the combination of genetic mutations might increase the risk of the disease onset or compound its severity.

This review aimed to investigate the most feasible IRD genetic screening method for a developing country such as Indonesia.

MATERIALS AND METHODS

Systematic searching for literature citations in this review was conducted in July 2022 with Boolean operators using terms 'inherited retinal dystrophy', 'inherited retinal dystrophies' and 'genome sequencing', 'whole genome sequencing', 'wide genome sequencing', 'whole exome sequencing', 'targeted sequencing', 'panel sequencing'; 'next generation sequencing' through the PubMed and Google Scholar databases. The screening procedure allowed the authors to exclude the less suitable references, i.e. systematic review, book chapters, comments and not genetic study. A flow diagram of the systematic search was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

The pooled detection rate of each genetic tool was analysed using the meta-regress protocol in the R program. The novel mutation discovery of each genetic tool each year was tabulated on a graph.

RESULTS

Suitable Genetic Testing Instrument

After conducting the manuscript screening, the eligible papers were tabulated and filtered (Figure 1). First, from matched 451 papers, duplication (n=6), and not original articles i.e. reviews, book chapters, and comments (n=238) were excluded. Secondly, the original article, but not a human genetics study was excluded (n=12). Third, papers with missing data or reports not retrieved (n=12) were excluded. Lastly, the paper does not elaborate on the number of solved and unsolved cases (n=12) and shows a high deviation (n=134) not included in the meta-analysis.

In the current review, five sequencing methods performances were compared, i.e. microarray, WGS, Sanger sequencing, panel sequencing, and WES. The detection rates of those five methods were 0.35, 0.39, 0.44, 0.55 and 0.65, respectively. In this analysis, WES has the highest positive rate.

Astuti performed studies using microarray sequencing in 2016,[8] Cauwenbergh in 2017,[9] and Barandika in 2015[10] with a total of 173 samples. Among those three, Barandikaa's study which tested 76 samples, had the highest detection rate of 0.32 with a weight of 44.5%.

The Sanger method sequenced a total of 498 samples with a detection rate of 0.44. The largest detection rate in the Ramkumar's in 2017[11] with 225 samples was 0.40 with a common weight of 45.7%.

The WGS method, with a total of 324 samples from the one conducted by Biswas in 2021,[12] Carss in 2017,[13] and Numa in 2020[14] showed a random effects model of 0.39. In this review, it was found that the WGS method in Numa's study with 171 samples had the most accurate diagnostic rate of 0.26 with a random weight of 38.5%.

Of the 37 analysed studies, 16 of them used the panel sequencing method, with a total of 4,350 samples having a detection rate of 0.55. Carss in 201713 used 722 samples with a detection rate of 0.56 and a common weight of 16.7%. Seven studies using the WES method with a sample size of 1374 had a detection rate of 0.65. Whelan, in 2020[15] with a sample size of 710, had the highest detection rate of 0.70 with a random weight of 27.4%.

After conducting correlation testing, the best pooled diagnostic rate for IRD was WES (0.66) then, followed by panel sequencing (0.55). The microarray, Sanger and WGS yielded less than 0.5 diagnostic rates.

Novel Mutation Discovery

Genetic variants associated with IRD are growing as novel mutations are discovered until 2021 (Figure 4). Panel-seq was the top contributor in 2019 with 384 new variants, surpassing WES by 175.

One, 156, four and one novel mutation was discovered by using microarray in 2007, 2011, 2015 and 2020, respectively. Five, one, one, and 42 novel mutations were identified by using WGS in 2017, 2018, 2020, and 2021, respectively.

However, the number of novel mutations discovered from all devices after 2019 decreased each year.

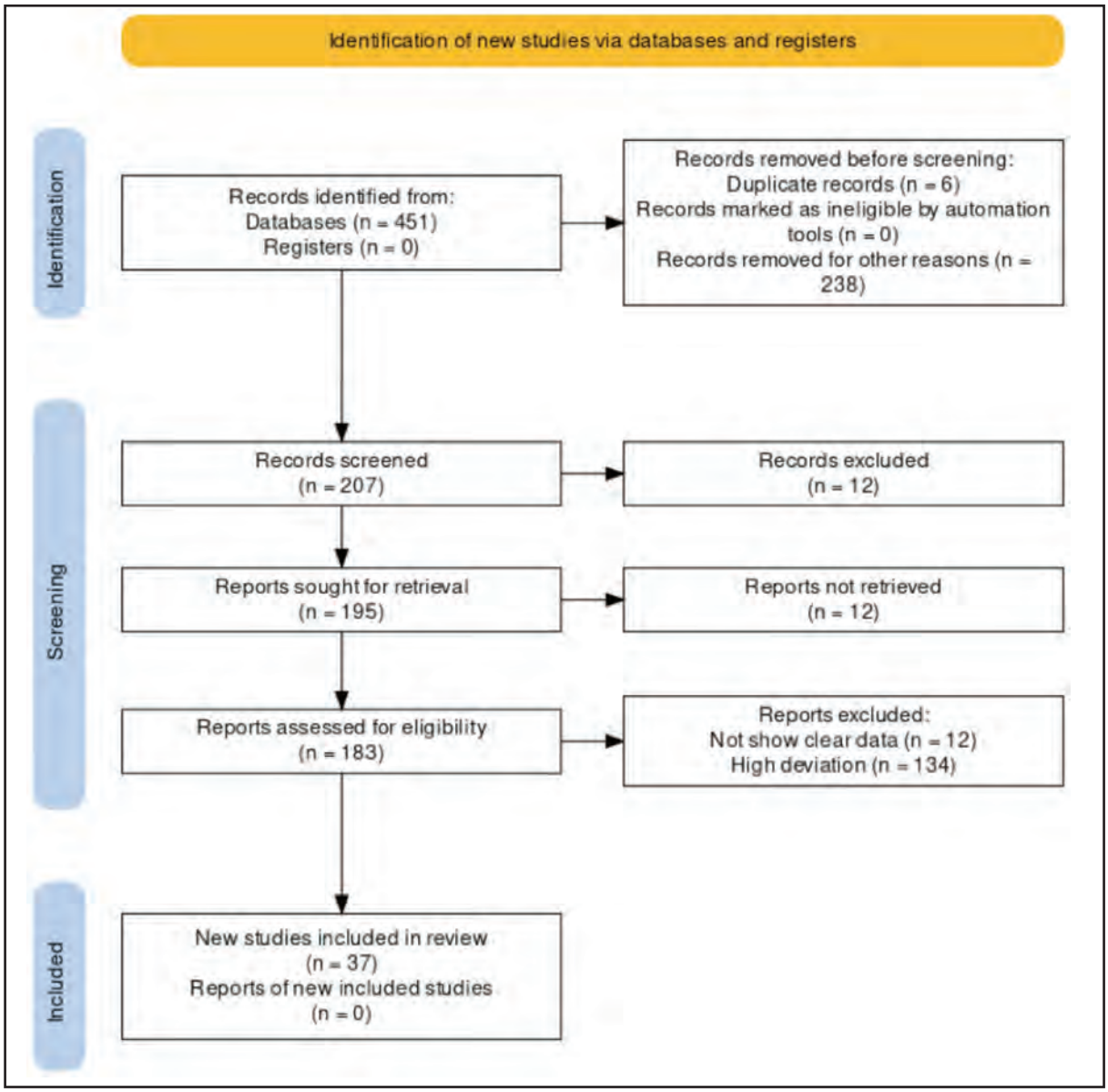

Fig. 1: The searching strategy for the review of inherited retinal dystrophy genome sequencing. After filtering the manuscripts, 37 eligible records were summarised (Table I).

DISCUSSION

The Genetic Testing Choice of IRDs

Whole exome sequencing shows a higher diagnostic rate than panel-seq in this review. The main reason was that the number of genes and variants included in each panel-seq used in each research varied. That widened the discrepancy in the diagnostic rates. On the other hand, WES included all variants and genes at the exome region by default. This created similar results to all WES research findings, which also yielded high diagnostic rate.

Although panel-seq on most research plans was placed as the first tier, this strategy did not lower the WES detection rate. The remaining unsolved IRD cases that underwent WES have yielded a good diagnostic rate. Consider using WES in research if cases remain unsolved by panel-seq.

The Sanger sequencing, as expected, had a low diagnostic rate as the narrowest type of testing. The success of Sanger sequencing depends on how specific the IRD was characterised and the loci target. Typical Sanger sequencing can read 300 nucleotides long, so the target genomic location is selected carefully. The monogenic or/and well-defined aetiology of a specific sub-type of IRD phenotype can be 100% identified by the Sanger sequencing approach.

On the other hand, a low diagnostic rate of WGS was unexpected. Theoretically, the resolving power of WGS was higher than WES, but in this review, did not yield a comparable diagnostic rate. The main concern was the last preference of using WGS in most of the research. The pooled unsolved cases from other instruments (including WES) and the complex and unclear phenotypes of IRD cases were difficult to solve even using WGS. The number of cases that

Table I: The filtered records included in the meta-analysis

No	authors	Sequencing Technology	Diagnosis Rate	Number of case
1	henderson 2007	microarray	0.44	153
2	Jin song 2011	microarray	0.79	19
3	Barandikaa 2015	microarray	0.32	76
4	Astuti 2016	microarray	0.38	40
5	Cauwenbergh 2017	microarray	0.37	57
6	Martin-Merida 2019	microarray	0.11	721
7	Neveling 2012	Panel seq	0.47	234
8	Li Zhao 2015	Panel seq	0.6	82
9	Consugar 2015	Panel seq	0.51	192
10	Zhongqi 2015	Panel seq	0.49	105
11	Patel 2016	Panel seq	0.62	292
12	Ellingford 2016	Panel seq	0.5	537
13	Carss 2017	Panel seq	0.56	722
14	Haer Wigman 2017	Panel seq	0.49	299
15	Handong dan 2019	Panel seq	0.57	76
16	Jiman 2019	Panel seq	0.52	106
17	Panfeng Wang 2019	Panel seq	0.52	568
18	Mun?oz 2020	Panel seq	0.62	172
19	Sheck 2020	Panel seq	0.59	488
20	Duzkale 2021	Panel seq	0.61	46
21	Maggi 2021	Panel seq	0.58	119
22	Ta-Ching Chen 2021	Panel seq	0.57	312
23	Sullivian 2013	Sanger seq	0.52	170
24	Astuti 2016	Sanger seq	0.41	64
25	Ramkumar 2017	Sanger seq	0.4	225
26	Collison 2019	Sanger seq	0.33	39
27	Weisschuh	wes	0.62	47
28	Riera 2017	wes	0.71	59
29	Bryant	wes	0.64	69
30	Whelan 2019	wes	0.7	710
31	Ahra Cho 2020	wes	0.57	250
32	Belal Azab 2021	wes	0.71	55
33	Yoon-Jeon Kim 2021	wes	0.6	184
34	Bujakowska 2016	wgs	0.18	28
35	Carss 2017	wgs	0.31	45
36	Numa 2020	wgs	0.26	171
37	Biswas 2021	wgs	0.56	108

underwent WGS was also lower than other instruments, making the statistics poor.

Microarray was similar to panel-seq in terms of variant customisation. Users can choose which variant and its number is included in the microarray. This makes the microarray detection rate also varied in each research. The number of studies using microarray was also limited. The reviews published during the 2000s suggested that microarray was less suitable than NGS-based methods for genotyping,16-18 which affects scientist preference.

The similarity between microarray and WES in various studies should yield a comparable diagnostic rate. However, the result of the pooled diagnostic rate of the microarray was lower than panel-seq.

The Availability and Pros-Cons of the Sequencing Platform for Highly Covered IRDs Mutation

The advanced technology has been developed to enhance the IRD genetic landscape. The number of reported loci was high, including the novel mutations. After the exhaustive use of wide genome analysis, the number of novel mutations should decrease every year.

To date, 4,798 discrete variants and 17,299 alleles were reported by Schneider in 2022.[19] Most variations are located at ABCA4 (24.8%) followed by USH2A (14.6%). Even the elusive cases of IRD were caused by a limited number of genes. So, a powerful panel-seq must be developed to substitute WES to cover all possible genetic causes of IRDs.

IRD populational genetic research in Indonesia or Southeast Asia was limited, so the current data cannot be directly interpreted for policy in Indonesia. However, the data still could suggest the most suitable genetic testing.

The prevalence of IRD and the list of common causative mutations in Indonesia remains unknown, but the total number of cases is expected to be high. The list of mutations included in the Panel can be determined only if the prevalence of causative mutations in the Indonesian population is known.

The utility of WES is no longer important if most of the associated genes were mapped. The more suitable genetic testing will be panel-seq. But at the time of developing a panel for genetic testing, the rate of new mutation discovery must be already low or not detected.

The acquired records were analysed further to pool the diagnostic rates of each technology (Figure 2).

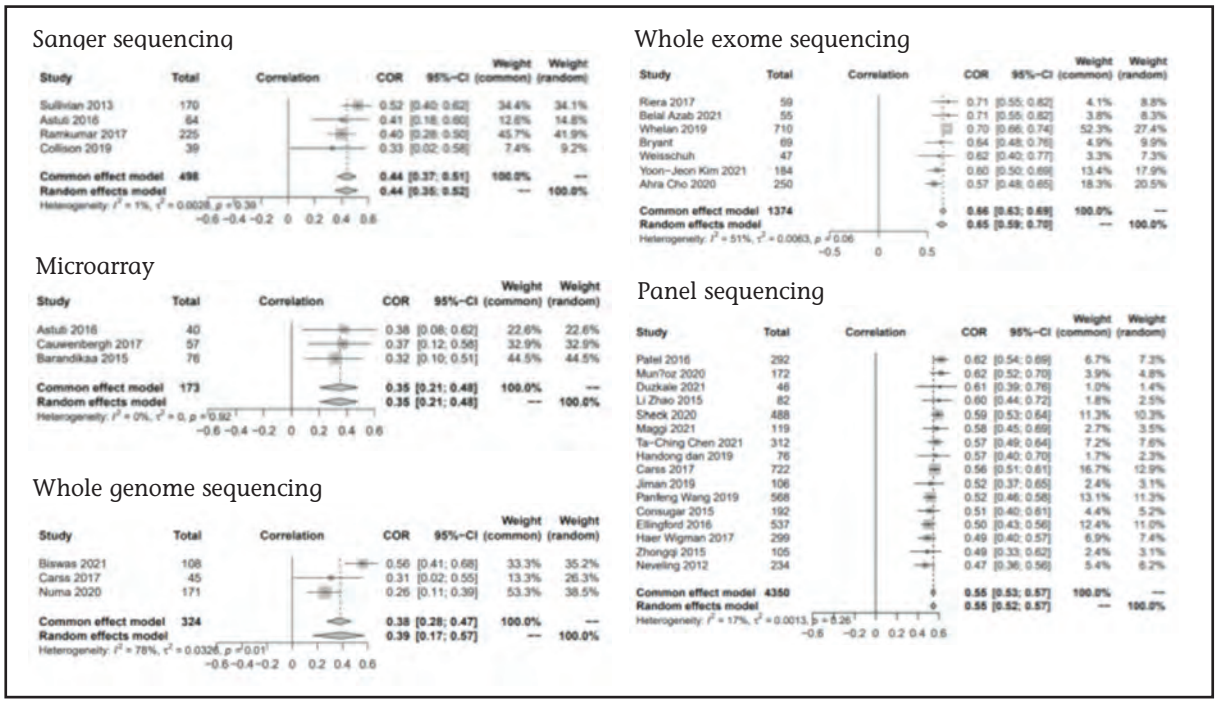

Fig. 2: Forrest Plot showing the diagnostic rate comparison of Sanger sequencing, microarray, and Next generation sequencing technologies (panel-seq, whole exome, whole genome). The whole exome yielded the highest detection rate.

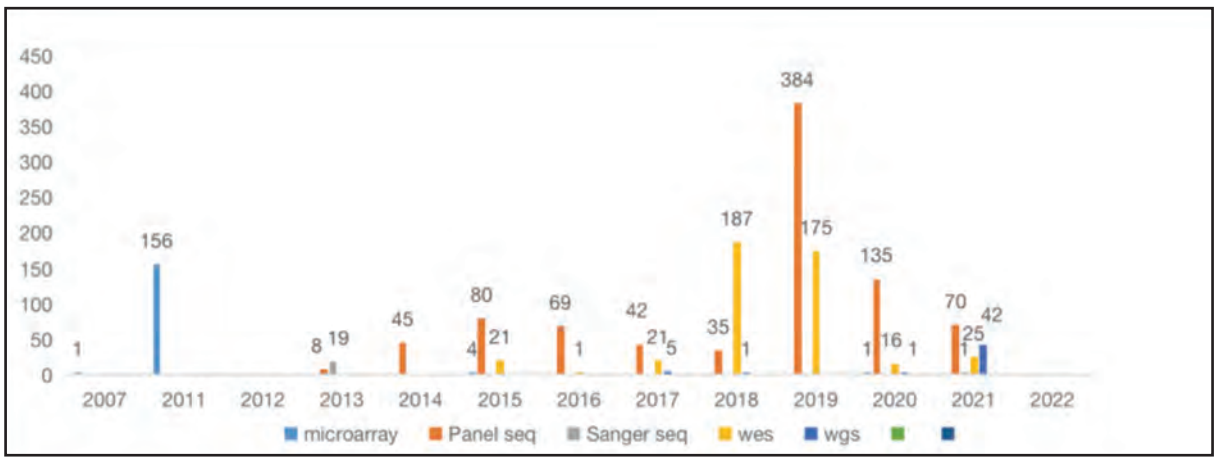

Fig. 4: The novel mutation discovery of inherited retinal dystrophy (IRD) year 2007 - 2022 by microarray, Sanger, panel, whole exome sequencing (WES), and whole genome sequencing (WGS).

The current running cost of WES was much higher than panel-seq as the routine diagnostic tool especially in Indonesia. The output data of panel-seq was also smaller than WES. It takes less effort to interpret data. So, panel-based sequencing costs will be more economical than WES. Routine diagnostics should minimise the laborious data analysis by utilising an automated pipeline.

CONCLUSIONS

Currently, the most suitable first-tier genetic testing for patients with IRD was whole exome sequencing for most IRD cases. However, for feasible genetic testing in the future, the first tier of genetic testing should be panel-seq.

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