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Next-generation whole-exome sequencing contribution to identification of rare autosomal recessive diseases

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A rare disease is any disease that affects a small percentage of the population. In the European Union a disease is defined as rare if it affects less than 1 in 2,000 people. Despite a small percentage of affected people by one disease, the total number of rare diseases is estimated to be around 7,000–8,000, thus, because of their large number they have an impact on many people and even 30 million of European Union citizens may be suffering from them. Research of rare diseases may help to explain their mechanism or to develop more advanced diagnostics. Classical strategies for studies of rare autosomal recessive diseases encounter with additional problems (multiple genetic variants, de novo mutations, extremely rare cases) that make these strategies not enough effective. Next generation whole-exome sequencing (WES) opened a new page in Mendelian disease gene discovery – enabling to study autosomal recessive diseases in a new way. During 3 years of WES usage many novel mutations of autosomal recessive disease genes were discovered.

Key words: rare autosomal recessive diseases, whole exome sequencing, syndromes

INTRODUCTION

A rare disease is any disease that affects a small percentage of the population. In the European Union, a disease is defined as rare when it affects less than 1 in 2,000 people. The number of the rare diseases is estimated to be about 7,000–8,000 and 1,139 of them have been characterized as recessive. Approximately 80% have a defined genetic basis. Despite low frequency of diseases, because of their great number, even 30 million European Union citizens may be affected by rare diseases (1, 2). Considering this, research of these diseases has great importance and study of them may help to explain the mechanisms of diseases and help to develop more advanced diagnostics.

Classical strategies to identify the cause of Mendelian diseases rely on using linkage analysis, homozygosity mapping, and association analysis.

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Linkage analysis is family-based approach that was a great tool for Mendelian disease discovery and has a relatively long history of successful detection of the rare Mendelian diseases such as Huntington's disease and cystic fibrosis (3). However, some of the rare Mendelian diseases can be caused by multiple genetic variants as well as by new de novo mutations which, according to Vissers (4), can be linked to 7 of 10 patients of mental disability – in these cases linkage analysis is not effective.

Autozygosity mapping has been a good method for the identification of the autosomal recessive disease genes. However, that approach is limited by the unavailability of suitable consanguineous families' genealogies. While rare autosomal recessive diseases are overrepresented in consanguineous families, a significant proportion of affected patients nonetheless originate in families where the parents are apparently unrelated (5). So, even if there is a successful history of the rare disease identification with classical approaches, it encounters with problems like multiple genetic variants, de novo mutations, extremely rare cases that make these strategies not enough suitable for disease study. A new turning point was appearance of the next-generation sequencing (NGS) technologies.

NGS technologies are a powerful tool for identification of genetic disease causes. These technologies enable the whole genome and exome sequencing to avoid homozygosity mapping, prioritize candidate genes and to proceed directly to the examination of the list of variants. That gives enough insight to "pinpoint" the mutation responsible for a disease. At present several national projects using NGS are released in Canada, Korea, UK, USA, and other countries (6, 7).

Disease gene identification challenge using NGS technology changes to interpretation challenge, because using it researchers obtain a sizeable amount of variation data that must be thoroughly filtered. This part of NGS is the most difficult one and requires efficient tools for filtering and interpreting results of sequencing (8).

Most of published whole genome studies have been focused mainly on the coding part, which is only about 1.5 percent of the whole genome, because of knowledge that roughly 85% of the known genetic causes of Mendelian disorders affect the protein coding regions (8, 9). The term 'exome' can be defined as a coding part of genome – all exons. Whole exome sequencing (WES) is an effective strategy for selective sequencing of only coding regions of genome (9). Advantage using WES is because of two reasons: firstly, there are extremely rare diseases with only few affected individuals and families per disorder, which result in underpowered analyses and / or large regions under the linkage peak(s); secondly, despite that these disorders are rare, the causal mutations show the large phenotypic effect (2, 8).

Although many different methods for targeted capture have been described, only few have been extended to target the human exome. These high-throughput sequence capture methods are based on hybridization, for example, array-based hybridization or liquid-based hybridization depend on technology that is used (10).

DISCUSSION

Initial exome sequencing was made by Sarah Ng and colleagues in 2009, when gene of the rare autosomal recessive genetic disorder, Miller syndrome, was found (11). This new approach has opened a new revolutionary page of opportunities for the rare disease research and diagnostic. During brief 3 years of WES usage a considerably high number of novel recessive disease genes have been discovered. That includes all types of inheritable rare diseases – autosomal recessive, autosomal dominant, X-linked. WES allowed finding genes that have an autosomal dominant pattern in diseases such as Kabuki, Schinzel-Giedion, Hajdu-Cheney syndromes, paroxysmal kinesigenic dyskinesia, primary lymphoedema, high myopia, dilated cardiomyopathy, autism, and others (12–16).

New X-linked disease causing genes such as TARP syndrome, leucoencephalopathy or X-linked intellectual disability were also discovered (17–19). Using exome sequencing technique mutations were found in mitochondrial DNA genes such as *MRPL3* (mitochondrial cardiomyophathy) or *AARS2* (infantile mitochondrial cardiomyophathy) (13, 20). That shows a great impact of WES on rare disease diagnostics. The autosomal recessive diseases especially benefit from this new method, and this review concentrates on such diseases.

In diseases research, the best way to carry out research is by combining few methods. In case of

rare diseases, many authors combine homozigosity mapping and exome sequencing. In disease studies, exome sequencing is used both as a basis research and as a way to confirm that gene mutation actually causes a disease. Combined use of exome sequencing and homozygosity mapping was applied in the study of Van Den Ende-Gupta syndrome, Joubert syndrome and other disorders (21–23).

One of WES advantages is that disease genes may be identified using a very limited number of patients – they may be identified even from only one patient. For instance, polycystic kidney disease's genetical cause was identified from only one 5-year-old young boy who had this disease. With the help of exome sequencing, two heterozygous *PKHD1* gene mutations were found, which lead to substitution of an asparagine for an aspartate, and that is the cause of disease. In this study, the researchers combined both, family and population, strategies (24). Another example, a rare recessive *FLVCR2* gene mutation that causes Fowler syndrome and is associated with progressive destruction of central nervous system tissue, was found out by testing only two patients (25). Ability to identify disease causing genes by exome sequencing only from a single or small number of affected individuals has considerable importance, as some rare diseases are so rare that it is difficult to find enough patients for that disease research.

As it was mentioned, since November 2009, exome sequencing has led to significant progress of rare autosomal recessive Mendelian disease identification. In the Table, these diseases are divided into vision and hearing; skeletal, teeth and skin; ciliopathic; nervous system and neuromascular as well as immunological groups.

In a short period of time, WES made a huge impact on research of ciliopathies – an emerging class of human genetic disorders that is caused by defects in cilia. These defects in cilia are associated with a range of human diseases, such as primary ciliary dyskinesia, hydrocephalus, polycystic liver and kidney disease, and some forms of retinal degeneration (26). Using WES, mutations that cause ciliopathy diseases were found: Van Den Ende-Gupta, Sensenbrenner, Joubert, Bardet-Biedl syndromes, Leber congenital amaurosis, polycystic kidney disease, primary ciliary dyskinesia (21–24, 27–31). Exome sequencing effectively confirmed that the nonsynonymous mutation chr22:19115386 C>T in the *SCARF2* gene, which is expressed during development, is responsible for Van Den Ende-Gupta syndrome that affects multi-system (21). Also there were studies where exome sequencing was applied to reveal genes causing Joubert syndrome – a neurological, ciliopathic disorder manifested by psychomotor retardation, hypotonia, and ataxia. In 2010, Edvardson (22) found mutation in the *TMEM216* gene related to Joubert syndrome type 2 in Ashkenazi Jews, and in 2012 Srour (23) found other mutations in *C5orf42* gene that also cause Joubert syndrome in the French Canadian population. An autosomal-recessive disease that is characterized by sagittal craniosynostosis and facial, ectodermal, and skeletal anomalies is known as Sensenbrenner syndrome. Gilissen and colleges (27) sequenced exomes of 2 patients and indentified compound heterozygous mutations in exon 2 of the *WDR35* gene, causing Sensenbrenner syndrome. Another disease's gene related with ciliopathy and affecting multi-system was identified in 2012. This disease is Bardet-Biedl syndrome and it is caused by a homozygous 5 bp deletion in the *LTZFL1* gene (28). Whole-exome sequencing performed by 2 different scientist teams showed that *NMNAT1* gene mutation is causing ciliopathic disease Leber congenital amaurosis (29, 30). Polycystic kidney disease and primary ciliary dyskinesia are also ciliopathic diseases that were studied with the help of whole exome sequencing (24, 31).

Exome sequencing also has some impact on vision and hearing diseases studies. Stargardt macular dystrophy, Leber congenital amaurosis and retinitis pigmentosa are diseases that are related with vision disorders and all of them were successfully analyzed by whole exome sequencing (29–30, 32–34). Stargardt macular dystrophy is an inheritable degeneration disease that causes progressive vision loss. Whole exome sequencing revealed 7 diseases likely causing variants across four genes, providing a confident genetic diagnosis in six previously uncharacterized participants. There were identified four previously missed mutations in the *ABCA4* gene across three individuals. Also, mutations were identified in *RDS/PRPH2, ELOVL,* and *CRB1* genes that are also likely to cause this disease (32). Retinitis pigmentosa (RP) is a heterogeneous group of

Table. Autosomal recessive disorders that were studied by using exome sequencing

Disease	Primary affected systems	Location of mutation (gene)	Authors	Year
Vision and hearing diseases				
Stargardt macular dystrophy (STGD)	Vision	*ABCA4*	Storm et al. (32)	2012
Retinitis pigmentosa	Vision	*DHDDS* *CYP4V2*	Züchner et al. (33) Wang et al. (34)	2011 2012
Usher syndrome type 3	Vision, hearing	*ABHD12*	Eisenberger et al. (35)	2012
Nonsyndromic hearing loss	Hearing	*GPSM2*	Walsh et al. (39)	2010
Perrault syndrome	Hearing, genital	*HSD17B4*	Pierce et al. (36)	2010
Brown-Vialetto-van Laere syndrome	Hearing, muscle, central and peripheral nervous system	*SLC52A3* *(C20orf54)*	Johnson et al. (37) Haack et al. (38)	2010 2012
Skeletal, teeth, skin diseases				
Osteogenesis imperfecta	Skeletal	*SERPINF1*	Becker et al. (43)	2011
Skeletal dysplasia	Skeletal	*POP1*	Glazov et al. (41)	2011
3-M syndrome	Skeletal	*CCDC8*	Hanson et al. (42)	2011
Chondrodysplasia and abnormal joint development	Skeletal	*IMPAD1*	Vissers et al. (44)	2011
Miller syndrome	Skeletal, hearing	*DHODH*	Ng et al. (11)	2010
Progeroid syndrome	Skeletal, CNS*, hair, skin, eyes	*BANF1*	Puente et al. (57)	2011
Amelogenesis imperfecta	Teeth	*FAM20A*	O'Sullivan et al. (45)	2011
Kohlschütter-Tönz syndrome	Teeth, CNS	*ROGDI*	Schossig et al. (46)	2012
Peeling skin syndrome	Skin	*CHST8*	Cabral et al. (47)	2012
Kaposi's sarcoma	Skin	*STIM1*	Byun et al. (48)	2010
Ciliopathies				
Leber congenital amaurosis	Vision	*NMNAT1*	Perrault et al. (29) Chiang et al. (30)	2012 2012
Joubert syndrome type 2 (JBTS2) Joubert syndrome (JBTS)	CNS, skeletal, eyes, ears, kidney	*THEM216* *C5orf42*	Edvardson et al. (22) Srour et al. (23)	2010 2012
Bardet-Biedl syndrome	Skeletal, CNS, vision, kidney, liver, heart	*LZTFL1*	Marion et al. (28)	2012
Polycystic kidney disease	Kidney, liver, lungs, pancreas	*PKHD1*	Da et al. (24)	2012
Primary ciliary dyskinesia	Lungs	numerous	Berg et al. (31)	2011
Sensenbrenner syndrome	Skeletal, eyes, kidney, liver	*WDR35*	Gilissen et al. (27)	2010
Van Den Ende-Gupta syndrome	Skeletal, CNS, eyes	*SCARF2*	Anastasio et al. (21)	2010
Nervous system / Neuromascular diseases				
Nonsyndromic mental retardation	CNS	*TECR*	Caliskan et al. (49)	2011
Hyperphosphatasia mental retardation syndrome	CNS, skeletal, heart	*PIGV PIGO*	Krawitz et al. (50)	2010 2012
Congenital cerebellar ataxia	Central and peripheral nervous system	*GRM1*	Guergueltcheva et al. (51)	2012
Infantile onset spinocerebellar ataxia	CNS, hearing	*C10orf2*	Dündar et al. (52)	2012
Spinocerebellar ataxia with psychomotor retardation	Central and peripheral nervous system, muscle	*SYT14*	Doi et al. (53)	2011
Progressive external ophthalmoplegia	Central and peripheral nervous system, muscle, heart	*RRM2B*	Takata el al. (40)	2011
Lethal congenital contractural syndrome Type 4 (LCCS4)	CNS, muscle, skeletal	*MYBPC1*	Markus et al. (54)	2012
Fowler syndrome	CNS	*FLVCR2*	Lalonde et al. (25)	2010
Immunological diseases				
Autoimmune lymphoproliferative syndrome	Blood	*FADD*	Bolze et al. (55)	2010
Aplastic anemia	Blood	*MPL*	Walne et al. (56)	2012

* CNS – central nervous system.

progressive retinal degenerations. Its symptoms include night blindness, tunnel vision and bone-spicule pigmentation in retina. Recently, it was known that over 50 genes can cause RP, but that explains no more than half of the clinical cases. The rise of exome sequencing could give a new insight into retinitis pigmentosa. Good examples are Wang and his colleagues' research in a large Chinese family that allowed finding retinitis pigmentosa causative mutations in the *CYP4V2* gene (33), and Züchner and his team's work with the Ashkenazi Jewish family, which showed that the *DHDDS* gene also harbors retinitis pigmentosa causing mutation (34).

Hearing diseases were also studied by exome sequencing including Usher, Perrault and Brown-Vialetto-van Laere syndromes, nonsyndromic hearing loss (11, 35–39). Usher syndrome is a retinitis pigmentosa syndromic form when a patient not only has a condition of vision loss, but also has hearing loss. With the help of homozygosity mapping and next-generation targeted exons sequencing in the Lebanese family which has Usher syndrome type 3 phenotype, causative mutation was found in *ABHD12* gene (35). Perrault and Brown-Vialetto-van Laere syndromes are two more examples of hearing disorders investigated by exome sequencing. With the help of WES causative mutation in Perrault syndrome was identified in the *HSD17B4* gene (36), and in Brown-Vialetto-van Laere syndrome such mutation was revealed in the *SLC52A3* gene (37–38). Nonsyndromic hearing loss was also studied using WES (39).

WES also allowed finding disease genes whose products are functioning in mitochondria, but are encoded by nuclear genes. Such situation was determined for the *RRM2B* gene, associated with autosomal recessive progressive external ophthalmoplegia. The *RRM2B* gene encodes a small subunit of ribonucleotide reductase small 2-like protein p53R2 which plays the essential role in the maintenance of mtDNA (40).

With the aid of WES, many diseases that affect bones, teeth and skin were studied. That includes skeletal dysplasia, 3-M syndrome, osteogenesis imperfecta, chondrodysplasia and abnormal joint development, Miller syndrome – bone diseases; amelogenesis imperfecta, Kohlschütter-Tönz syndrome – teeth diseases; as well as skin diseases – peeling skin syndrome, Kaposi's sarcoma (11, 41–48). Skeletal dysplasia, that commonly is called dwarfism, is a group of disorders characterized by abnormalities of cartilage and bone growth. When whole-exome sequencing was applied to a family of two healthy parents and two affected children with skeletal dysplasia, two novel compound heterozygous loss-of-function mutations in the *POP1* gene were found and identified as disorder causative mutations (41). Another investigation using WES related with skeletal dysplasia was held by Hanson et al. (42). Mutation in the *CCDC8* gene was idenfitied as a cause of 3-M syndrome that is known as a syndromic form of skeletal dysplasia. One more genetic bone disorder is osteogenesis imperfecta. Patients with such disorder have very easily breaking bones. With the help of WES, it was determined that four unrelated individuals had *SERPINF1* gene mutations as an osteogenesis imperfecta cause (43). As for teeth disease, amelogenesis imperfecta, characterized by abnormal enamel formation, and the related Kohlschütter-Tönz syndrome are examples of WES studied teeth diseases. Mutation in the *FAM20A* gene related with amelogenesis imperfecta (45) and the *ROGDI* gene mutation related with Kohlschütter-Tönz syndrome were found (46). One of successful examples for WES applying to the skin diseases is peeling skin syndrome, genetic disorder characterized by continual peeling of the skin. By homozygosity mapping and whole-exome sequencing a novel homozygous missense mutation was identified within the the *CHST8* gene (47). Another example is finding that the gene *STIM1* is related with Kaposi's sarcoma, which is skin cancer (48).

An important disorder group represents diseases that cause damage of the nervous system. In this category the whole-exome sequencing also keeps pace with other disorder groups and there is already not significant number of diseases inheritable in autosomal recessive or dominant manner and studied using WES. As for recessive diseases causing damage in the central nervous system and studied by WES, nonsyndromic mental retardation and hyperphosphatasia mental retardation syndrome are fine examples (49, 50). In these both diseases damage of CNS causes mental retardation. In 2011 using WES, Caliskan and colleagues found that mutation in the *TECR* gene causes nonsyndromic mental retardation (49). Krawitz using WES performed an exhaustive inquiry of hyperphosphatasia mental retardation

syndrome and found mutations in two genes *PIGV* and *PIGO* causing this disease (50). Disorders related with ataxia were also studied with the help of WES. Ataxia develops as a consequence of cerebellum degeneration responsible for control of muscle coordination. With the aid of WES, different ataxia types were investigated – congenital cerebellar ataxia that is caused by mutation in the *GRM1* gene, infantile onset spinocerebellar ataxia caused by mutation in the *C10orf2* gene and spinocerebellar ataxia with psychomotor retardation caused by *SYT14* gene's mutation (51–53). Previously mentioned progressive external ophthalmoplegia as well as lethal congenital contractural type 4 and Fowler syndromes also belong to the nervous system diseases group analyzed with WES (25, 40, 54).

Refering to autosomal recessive manner inheritable immunological diseases studied by whole-exome sequencing, autoimmune lymphoproliferative syndrome and aplastic anemia could be mentioned. Autoimmune lymphoproliferative syndrome is an immune system disorder characterised by too large number of lymphocytes production followed by numerous autoimmune problems. Previously it was known that *FASL* and *FAS* gene mutations cause this disorder, but with combination of whole-exome sequencing and genome-wide linkage analysis it was shown that lymphoproliferative syndrome also develops after mutation in the *FADD* gene (55). Another disease, aplastic anemia, when bone marrow fails to make enough blood cells, was studied using exome sequencing in the Tunisian family with 2 affected children. In both patients with aplastic anemia, the sequencing data showed the c.1248 G>A mutation in the *MPL* gene (56).

CONCLUSIONS

In conclusion, the whole-exome sequencing, during its short period of existing (three years), was successfully applied to many different rare autosomal recessive diseases. Such variety of diseases studied by WES are referred to in the present review. The number of studied diseases is further growing very fast; therefore WES will take an important place in rare disorders research. Further using of whole-exome sequencing and fast increasing information on the rare diseases allow prognosticating a wide application of WES in population studies.

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GENŲ, LEMIANČIŲ RETAS AUTOSOMINES RECESYVINES LIGAS, TYRIMŲ REZULTATAS PAGAL EGZOMO SEKOSKAITĄ

Santrauka

Reta laikoma ta liga, kuria serga tik nedidelė populiacijos dalis. Europos Sąjungoje reta liga laikoma tada, kai ja serga mažiau nei 1 iš 2 000 žmonių. Ir nors nuo vienos retos ligos kenčia nedaug žmonių, tokių retų ligų yra daug. Manoma, kad tokio pobūdžio ligų yra apie 7 000–8 000. Tad vien Europos Sąjungoje tokiomis ligomis sergančių asmenų priskaičiuojama per 30 milijonų. Toks didelis sergančių asmenų skaičius verčia domėtis ir tirti retas ligas, ir tai gali padėti išaiškinti šių ligų atsiradimo priežastis bei sukurti naujus, tobulesnius jų diagnozavimo būdus. Klasikinės paveldimų ligų tyrimo strategijos susiduria su papildomomis problemomis (dauginiai genetiniai variantai, *de novo* mutacijos, ypač reti atvejai), ir tai daro šias strategijas nepakankamai veiksmingas. Naujos kartos sekoskaita atveria naują retų ligų tyrimų puslapį. Viso egzomo sekoskaita per trumpą gyvavimo laikotarpį sugebėjo nemažai prisidėti ieškant retų autosominių recesyvinių ligų genetinių priežasčių – per trejus metus buvo aptikta ir ištirta daug naujų mutacijų genuose, lemiančių šias ligas.

Raktažodžiai: retos autosominės recesyvinės ligos, viso egzomo sekoskaita, sindromai