

Principles of Genetic Testing for Dyslipidemia in Children

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ABSTRACT

The genetic causes of several dyslipidemias have been identified. Our knowledge of the role of genetics in disorders affecting lipid and lipoprotein metabolism continues to improve along with advancements in technology and access of testing. Genetic testing offers diagnostic confirmation of disease, risk stratification, the ability to identify at risk biologic relatives, and individualized treatment options. While currently underutilized, genetic testing will increasingly play a key role in the treatment and management of children with lipid disorders.

INTRODUCTION

In 2003, the cost of sequencing the first human genome was \$2.7 billion. This pioneering work paved the way for genetic testing to become a practical tool in clinical practice. In 2016, the cost of genetic testing had declined to < \$1,000. With cost continuing to decline, genetic testing is being utilized more frequently to help clinicians make informed decisions about clinical care. As genetic testing plays an increasingly important role in clinical management, it has become imperative that those who provide care for individuals with lipid and lipoprotein abnormalities understand the basic principles of genetic testing in

order to provide appropriate care and accurate counseling, especially for affected children.

Although often underutilized, genetic testing helps to identify variants that play a causal role in disturbances of lipid and lipoprotein metabolism. Despite the benefits, the decision to perform a genetic test in youth requires a thorough understanding of the utility of genetic testing, as well as the nuances associated with testing in this younger population. Are children able to understand the purpose of the test being recommended and the short- and long-term consequences potentially associated with genetic test results? What rights does the child have in deciding whether or not to undergo testing? While many excellent and comprehensive publications are available on the genetic causes of lipid and lipoprotein disorders, the goal of this chapter is to discuss basic concepts of genetic testing, assist providers in the use of genetic testing – including the use of genetic counseling and interpretation and effective communication of results, address special considerations for genetic testing in youth, and discuss future directions in the field of diagnostic genetics as it relates to pediatric lipidology.

WHY IS GENETIC TESTING IMPORTANT?

When correctly utilized and properly communicated, genetic testing has the potential to provide significant

benefits in both clinical management and patient education (1). Correct diagnosis of a genetic disorder can accurately assess risk and help inform clinical decision making for the child as well as affected and unaffected family members.

For example, FH, a common condition (1:220), significantly increases an individual's risk of premature cardiovascular disease (CVD) due to elevated levels of low-density lipoprotein cholesterol (LDL-C) (2). Although individuals with heterozygous FH have a variable phenotype, the presence of a genetic variant results in a significantly higher risk for developing CVD due to lifelong exposure of elevated levels of atherogenic LDL-C (3, 4). Because FH is inherited in an autosomal co-dominant manner, first degree family members of those identified with a causative FH variant have a 50% chance of being affected and are at increased risk for developing CVD. Genetic testing can assist in therapeutic decision making for the index case as well as other family members identified by cascade screening or previously known to have hypercholesterolemia (5).

Value to Youth

When considering FH, unique benefits exist in identifying a causative genetic variant of those under 18 years of age. While CVD-related events typically occur in adulthood, the presence of persistently elevated cholesterol levels from an early age leads to the formation of atherosclerosis, beginning in childhood (5), and plays a key role in CVD risk and progression (6). By identifying an at-risk child, properly assessing risk and initiating treatment, including early introduction of a heart-healthy lifestyle and appropriate lipid-lowering medications, risk can be dramatically reduced, with the goal of reducing or preventing future ASCVD-related events, such as a heart attack or stroke (7, 8).

Furthermore, when a child is identified with FH, reverse cascade screening can identify other affected family members. Because of its mode of inheritance, 50% of first-degree relatives of a child with genetically confirmed heterozygous FH are also affected, many often unaware of their condition, and therefore, not receiving lipid lowering medications (Figure 1) (9).

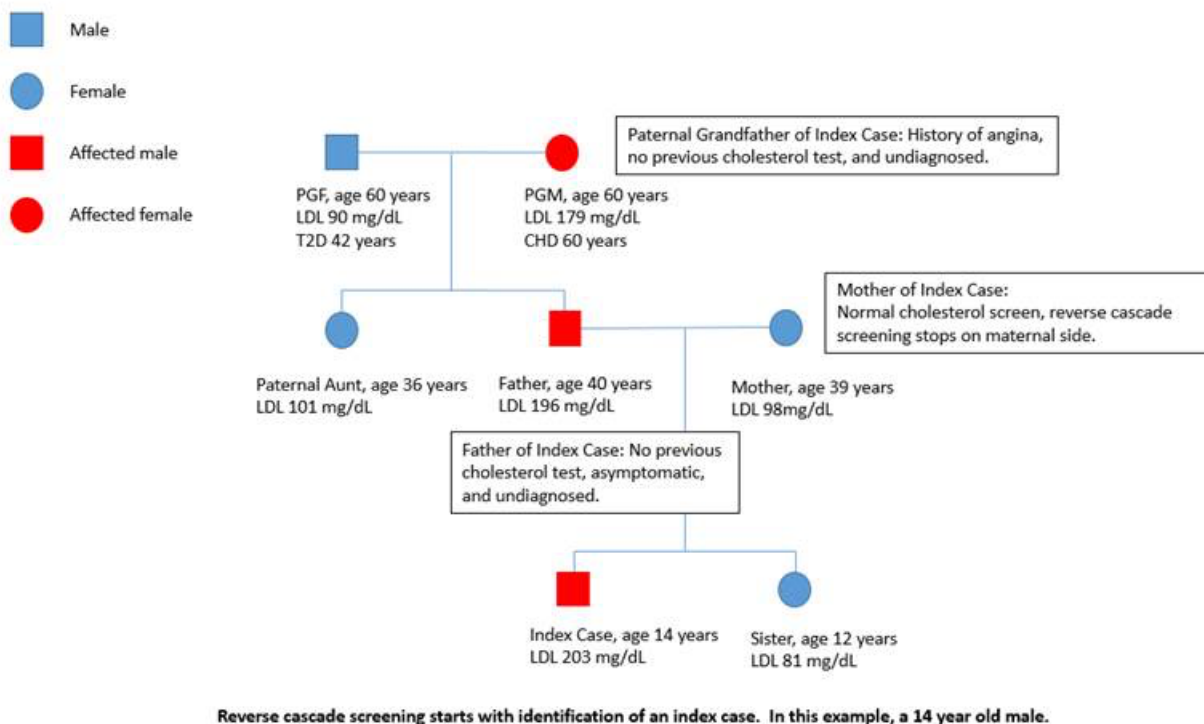


Figure 1. Sample pedigree from reverse cascade screening of proband. From *Journal of Pediatric Nursing*, 2019.

COMMON GENETIC TERMINOLOGY

Coverage: Number of genes sequenced.

Depth: Number of times each nucleotide within a gene is sequenced.

Exome: Part of the genome that consists of exons. The exome accounts for roughly 1% of the genome (Figure 2).

Exon: A segment of a gene that encodes a protein (Figure 2).

Genome: A complete set of genetic information that provides all necessary information required for a human to function (Figure 2).

Intron: A noncoding region of DNA, or a segment of DNA that does not encode a protein (Figure 2).

Single Nucleotide Polymorphism (SNP): A common (present in >1% of population), typically low effect variant, occurring at a single nucleotide in the genome (Figure 2).

Splicing: A process by which introns are removed from a transcript to produce mature RNA, made up of exons (Figure 2).

Variant: An alteration in the DNA nucleotide sequence. Variants can be benign, pathogenic, or of unknown significance.

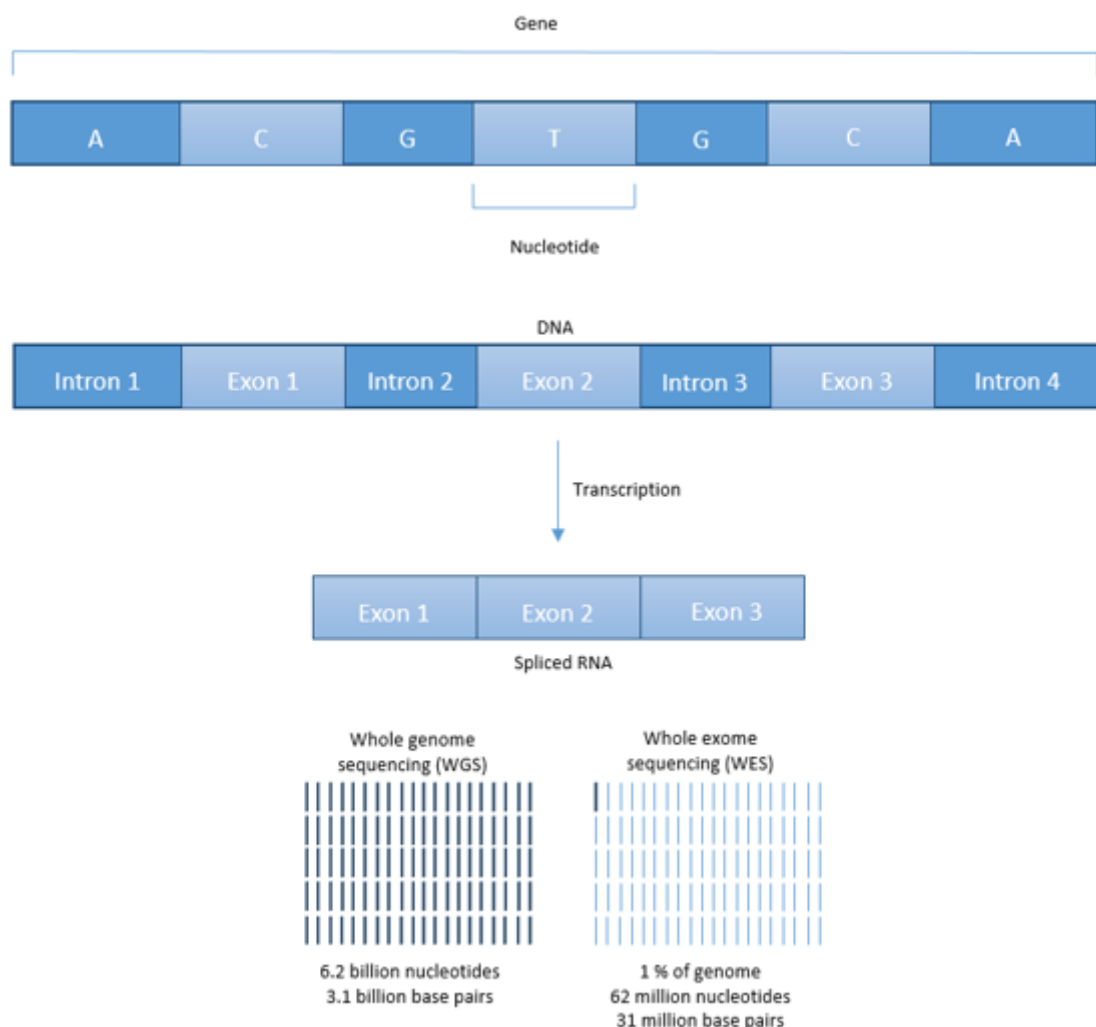


Figure 2. Visual depiction of a gene, nucleotide, introns and exons, splicing, and genome and exome sequencing.

AVAILABLE GENETIC TESTING

Targeted Panel

When considering conditions with known causal genetic loci, such as FH, targeted panels are often considered as a primary testing method. Four genes – *LDLR*, *APOB*, *PCSK9*, and *LDLRAP1* – are principally considered when identifying pathogenic variants

causing FH. While coverage is low (i.e., 4 genes), depth – depending on the performing laboratory – is high, often 100X up to 1,000X.

Targeted panels are most accurate when used to identify variants in exons and smaller deletions or duplications. Using a combination of next generation sequencing technologies, and Sanger sequencing and deletion/duplication analysis when necessary, analysis is performed to identify genetic variation often

with >99% sensitivity and specificity. Introns are typically not sequenced beyond +/- 10 to 15 exon flanking base pairs.

Whole Exome Sequencing (WES)

As NGS technologies continue to evolve and cost declines, sequencing DNA of higher volume has become more feasible. WES allows for sequencing of all protein coding regions of a person's genome, also known as the exome, along with flanking intronic regions. WES is often performed when the differential diagnosis is unclear or broad, or after a targeted genetic testing returns negative.

In the case of FH, WES can be helpful when no known variant is found in a traditional targeted panel. Several other conditions affecting lipid metabolism with known genetic variants – in *APOE*, *ABCG5*, *ABCG8*, *LIPA*, etc. – can produce a “FH phenotype,” in which conditions associated with variants in these genes create an overlap in elevated LDL-C levels with those seen in pathogenic FH variant carriers. Coverage in WES is high (i.e., 95 to >99% of the exome), while depth is often 20X up to 100X.

SECONDARY FINDINGS

It is important to note that targeted panels inclusive of candidate genes and WES have the potential of identifying unintentional or secondary findings. For example, certain variants in *APOE* are associated with a FH phenotype; however, other *APOE* variants are associated with a predisposition for Alzheimer's disease. When WES is performed, secondary findings for variants in gene sites unrelated to the condition under suspicion can occur. For example, WES ordered for suspicion of FH could return variants in *BRCA1/2* associated with a predisposition to develop breast or ovarian cancer and carry implications for other potentially affected family members. When secondary findings are identified, patients should be referred either to a geneticist or other relevant specialist. However, depending on the test ordered,

ideally directed by the patient's and provider's preferences, secondary findings can be excluded. Concerns about secondary findings can be alleviated by masking extraneous results.

SHOULD FAMILY MEMBERS BE TESTED?

Low or no cost genetic testing is sometimes offered to family members in an effort to both identify additional at-risk family members and help inform genotype/phenotype correlations for more accurate classification of gene variants.

INTERPRETING TEST RESULTS

How are Genetic Variants Classified?

Understanding a finding's genetic variant classification can be a daunting task. No standardization of classification is uniformly adhered to, with each genetic testing laboratory offering their own definition or algorithm for classification. This ultimately results in the potential for one laboratory to define a variant as benign, while another may define the same variant as pathogenic. To further complicate matters, classification for each variant is subject to change as new and additional data about the variant is considered (10).

Interpretation of a pathogenic classification is the most straightforward. In the case of FH, the observed variant is considered to be the cause of the phenotype, based on sufficient evidence of 1) the variant type, and 2) other individuals previously identified with the same variant.

Interpretation becomes complicated in the case of a variant of unknown significance (VUS) and those with negative results. When faced with a VUS, it is important to consider how important additional data is in determining a causal link between a VUS and the clinical condition. Fortunately, many, but not all, genomics companies offer first degree relatives genetic testing at low or no cost. Familial testing provides additional data to assist in 1) more accurate

classification of the finding in question, and 2) clinical judgement to more accurately guide health care decision making.

In the case of a negative result, it is important to understand any limitations that exist with the chosen test method. If a targeted panel for FH is ordered and a pathogenic variant is not identified 1) the test ordered may not include all known variant sites, 2) additional potential variants exist, and 3) additional testing (WES) may be helpful.

COMMUNICATING TEST RESULTS

What is the Role of a Genetic Counselor?

Given the complex nature of genetic testing as a diagnostic tool, genetic testing plays a crucial role in the patients understanding of risks and benefits of performing testing (11). Counseling should begin prior to testing being performed, and should continue after as a conversation with both the patient and their family.

Prior to testing, the patient should be informed of 1) the suspected condition and how genetics may play a role, 2) the possible benefits and risks of performing testing, and 3) the potential of discovering uncertain or secondary findings.

After completion, test results and interpretation of their impact on both direct patient care and family members should be discussed. If necessary, counseling for family planning and any further testing should be covered.

What is the Potential Impact of Genetic Testing Upon the Child? Family?

Proper genetic counseling and communication of test results provide the patient and family information of high utility, usually with minimal adverse impact (12). In 2017, using interviews of patients treated for FH who were the first to undergo genetically tested in their family, Hallowell et al. found testing was considered beneficial, as it provided patients with an origin of their disease and assessed their own and their family members' risk (13). The majority of parents of children with FH want their children to be tested (14) and children have been found to understand and articulate their understanding of testing being conducted (15). A majority of families do not report psychological problems due to a diagnosis of FH (16).

WHO TO TEST?

In addition to those with FH, the genetic basis for several other disorders affecting lipid and lipoprotein metabolism have been described (Table 1).

Table 1. Genetic lipid disorders. Modified from *Current Genetic Medical Reports*, 2016

Condition	Variant Site	Inheritance	Typical Lipid Effect
Abetalipoproteinemia	<i>MTTP</i>	Autosomal recessive	↓↓LDL-C
Homozygous hypobetalipoproteinemia	<i>APOB</i>	Autosomal codominant	↓↓LDL-C
Heterozygous hypobetalipoproteinemia			↓LDL-C
Chylomicron retention disease	<i>SAR1B</i>	Autosomal recessive	↓LDL-C

PCSK9 deficiency	<i>PCSK9</i>	Autosomal codominant	↓LDL-C
Familial combined hypolipidemia	<i>ANGPTL3</i>	Autosomal codominant	↓LDL-C
Homozygous familial hypercholesterolemia	<i>LDLR, APOB, PCSK9, LDLRAP11</i>	Autosomal codominant ¹ Autosomal recessive	↑↑LDL-C
Heterozygous familial hypercholesterolemia			↑LDL-C
Sitosterolemia	<i>ABCG5, ABCG8</i>	Autosomal recessive	↑LDL-C
Cerebrotendinous Xanthomatosis	<i>CYP27A1</i>	Autosomal recessive	↑LDL-C
Lysosomal acid lipase deficiency	<i>LIPA</i>	Autosomal recessive	↑LDL-C
Smith Lemli Opitz syndrome	<i>DHCR7</i>	Autosomal recessive	↓HDL-C
Tangier disease	<i>ABCA1</i>	Autosomal recessive	↓HDL-C
Fish eye disease	<i>LCAT</i>	Autosomal recessive	↓HDL-C
Apolipoprotein A-I deficiency	<i>APOA1</i>	Autosomal codominant	↓HDL-C
Scavenger Receptor B1 deficiency	<i>SCARB1</i>	Autosomal codominant	↑HDL-C
Cholesterol ester transfer protein deficiency	<i>CETP</i>	Autosomal codominant	↑HDL-C
Hepatic lipase deficiency	<i>LIPC</i>	Autosomal recessive	↑HDL-C
Familial chylomicronemia syndrome	<i>APOA5, APOC2, GPD1, GPIHBP1, LMF1, LPL</i>	Autosomal recessive	↑↑TG

WHAT'S NEXT?

Genetic testing in patients with lipid disorders may be considered in cases where diagnostic confirmation may benefit the patient through availability of or access to treatment, change in clinical management, or identification of other at-risk family members.

The progression of genetic testing has resulted in slowly changing the paradigm in clinical practice. Having most recently experienced the evolution of evidence-based medicine, we are entering an era of personalized medicine, and eventually, predictive medicine. In the coming years, existing methods and

results will become better understood, and additional testing will likely become more affordable, accurate, and widely used, leading to a potential shift in the clinical focus from phenotype to genotype.

Genomic Medicine

The current focus of genetic testing involves sequencing of exomes, accounting for only 1% of the genome. In contrast, whole genome sequencing (WGS) offers sequencing of both exons – protein encoding regions – and introns, containing regulatory information which controls exon splicing, transcription, and translation. Deep intronic variants are currently associated with over 75 genetic conditions (17).

Additionally, RNA testing potentially offers similar benefits to WGS without having to analyze such a large volume of data. RNA testing potentially identifies any errors, including intronic variants, leading to incorrect splicing or transcript sequence. In the realm of lipidology, those with FH caused by a variant affecting apolipoprotein B (apoB) may have the most to benefit from RNA testing. ApoB circulates in 2 forms: apoB48, produced by the small intestine, and apoB100, produced by the liver, the latter involved in LDL assembly and uptake by the LDL receptor. Both forms are encoded by a single *APOB* gene, which undergoes an RNA editing process, producing both forms (18). In the future, investigating transcription and translation of *APOB* may prove useful in determining etiology of disease in patients with a currently unidentified variant. However, RNA sequencing may be complicated due to the necessity of tissue specific extraction. In the case of testing *APOB*, liver would be required for appropriate sequencing.

Predictive Medicine

A significant portion of the general population, including those with a monogenic cause of FH, contain variants in genes associated with elevated cholesterol and CVD risk other than *LDLR*, *APOB*, *PCSK9*, and *LDRAP1*. SNPs are common variants of single

nucleotides in a number of “low effect genes,” or genetic locations that do not greatly affect the phenotype. However, when cumulatively expressed, cholesterol or triglyceride levels or CVD risk can be raised to levels of those with monogenic disorders. It has been suggested that the vast majority of those with elevations of LDL-C may be a result of polygenic disorders, resulting in an FH-like phenotype. Additionally, severe TG elevation has been attributed to rare, polygenic forms of FCS and multifactorial chylomicronemia syndrome, a disorder in which cumulative expression of TG elevating variants along with a secondary risk factor (i.e., poorly controlled diabetes, obesity, hypothyroidism, etc.) results in a TG elevation similar to that seen in those with FCS. SNPs serve as the basis for genome wide association studies and genetic risk scores which respectively aim to correlate common genetic variations with presence of disease, and define risk of an outcome defined by the presence of a specific genetic profile.

Polygenic risk scores employ algorithms that aggregate a profile of an individual's SNPs which have been associated with certain outcomes, including LDL-C or TG elevation, and CAD risk. Cholesterol and CAD risk scores are of increasing interest in clinical practice (19-23).

Screening and Preventive Medicine

Considering the future of current methodologies, genetic testing of youth and their parents has proven feasible and effective in the UK, and universal phenotypic screening of young children in the US is currently recommended (2, 24). FH also has potential to be a target for prenatal testing (25). By combining established universal phenotypic childhood screening (26) with reflex genetic and parental testing, the potential exists to identify every existing case of FH within one generation of testing. Subsequent targeted testing of affected patient's children would identify future cases.

SPECIAL CONSIDERATIONS FOR YOUTH

While benefits exist that are unique to a pediatric population, additional unique circumstances should be also be considered when testing a child for a condition in which disease onset occurs during adulthood.

Should Children be Given a Choice?

The American Academy of Pediatrics (AAP) advocates for youth to have an increasingly important role in their own health care decisions as they age and mature. From a legal perspective, virtually no legal rights exist, nor are protections in place, to ensure a child possesses autonomy in the decision making process of their own health care (27). The decision to include the child in the process is ultimately left to the child's parents and health care provider.

Should Testing be Deferred Until a Child is 18 Years-of-Age or Older?

In 2013, the AAP and American College of Medical Genetics (ACMG) released a joint policy statement on the use of genetic testing and screening of children (28), agreeing that the principle factor in determining whether to offer genetic testing should be the best interest of the child. In the case of FH, many feel that clear benefit exists in testing of children, as atherosclerosis can be reduced or prevented with early identification and treatment, ultimately reducing CVD risk.

Do the Results of Genetic Testing Create the Potential for Discrimination?

Once a child has undergone testing, results are entered into the patient's medical record. The Genetic Information Nondiscrimination Act (GINA) of 2008 protects individuals from discrimination in health insurance and employment based on genetic information. GINA provides no guarantee that 1) Health insurers will pay for genetic test or the medical care that a genetic test indicates are appropriate; 2)

Prevent revealing a test result or family history to an insurer to prove medical necessity; and 3) Protect against discrimination when applying for life insurance, disability insurance, or long term-care insurance. As children become adults, it is important to note that GINA does not apply to members of the United States military, veterans obtaining healthcare through VA, and the Indian Health Service.

In addition to perceived benefits, all potential risks must be considered and discussed before genetic testing of a minor is performed.

SUMMARY

Genetic testing offers 1) diagnostic confirmation; 2) enhanced risk assessment; 3) an ability to identify affected family members; and 4) the opportunity to individualized treatment options. Lipidiologists are encouraged to use this emerging technology judiciously, mindful of the unique needs of youth. In the near future, genetic testing will likely be used on a wide scale to screen children and family members at-risk of CVD, with the goal of primary prevention. Given its current trajectory, genetic testing is becoming increasingly critical in our ability to provide accurate risk assessment as well as age appropriate and timely intervention to help guide our efforts in educating and managing youth with disorders of lipid and lipoprotein metabolism.

RESOURCES

Select Laboratories Offering Genetic Testing for Dyslipidemias

Quest	Diagnostics:
https://www.questdiagnostics.com/	
LabCorp: https://www.labcorp.com/	
Ambry Genetics: https://www.ambrygen.com/	
Blueprint Genetics: https://blueprintgenetics.com/	
GeneDx: https://www.genedx.com/	
Invitae: https://www.invitae.com/en/	

The Genetic Information Nondiscrimination Act (GINA) of 2008

<https://www.eeoc.gov/laws/statutes/gina.cfm>

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