APPLICATIONS OF NEXT-GENERATION SEQUENCING — VIEWPOINT

Next-generation sequencing in the clinic: are we ready?

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Abstract | We are entering an era in which the cost of clinical whole-genome and targeted sequencing tests is no longer prohibitive to their application. However, currently the infrastructure is not in place to support both the patient and the physicians that encounter the resultant data. Here, we ask five experts to give their opinions on whether clinical data should be treated differently from other medical data, given the potential use of these tests, and on the areas that must be developed to improve patient outcome.

Q *Are clinical genomic data different from other medical test data? Should they be handled differently?*

Leslie G. Biesecker. Genomic test data (clinical genomes and exomes) are stupendously complex but so are a number of other complex medical tests. So, genomics is not different solely because of this complexity. But this similarity to other tests obscures two important aspects of genomic data requiring that a nuanced response to the second question is given. The first is that there are a few genes in a genome or an exome that are known counselling landmines (for example, genes involved in Huntington's disease and frontotemporal dementia[1]). It is an open question as to how we should deal with these genes in the context of genome and exome sequencing. Problems associated with testing these genes can be avoided by ensuring that the professionals who order, perform, analyse and interpret these genomes and exomes are trained and cognizant of the challenges that such testing entails and can manage the testing appropriately. Stumbling on these is inexcusable: these landmines are known, and they should be anticipated.

The second unique aspect of a genome or an exome test is the breadth of the testing. Even in this aspect, genomics is not truly unique. Ironically, the general physical examination comes closest to a genome or an exome in the breadth of what is being sought, what might arise 'incidentally' and the fact that it is difficult to consent a patient to the potential consequences of this evaluation. The irony is that it is the oldest test, and we are all quite comfortable with it. However, human beings, especially ones who are ill, are complex emotional creatures and are poorly equipped to absorb information regarding potential health threats on anything near the scale of a genome. At the same time, it is possible to extract a clinically useful subset of the data and to use it to modify the health care of a patient[2,3].

My conclusion is that clinical genomic data are the same as other complex medical data and need to be interpreted and delivered to the patient by professionals (typically, a molecular geneticist, a clinical geneticist and genetic counsellors) who have the skills to interpret these results in the context of the test methodology, the theoretical background of genetics, Bayesian reasoning and a myriad of other factors.

Wylie Burke. Clinical genomic data have the same purpose as other medical tests: to provide information to improve a patient's health care. In that sense, they are no different from other tests. However, clinical genomic data raise challenges that, while not unique, are different in scope. These challenges include: a high likelihood of incidental findings[4,5]; information relevant to family members; and findings that, by current standards of practice, call for pre-test counselling to ensure that the patient understands the nature of the information to be provided by testing, thus giving them the opportunity to decline testing. This third category includes information about carrier status[6] and about future health risks for which there is no treatment[7]. There is an urgent need for consensus development on the appropriate handling of each of these issues. We can anticipate, however, that practice standards for genomic data will incorporate lessons derived from the history of genetic testing[8]: privacy of health records should be assiduously protected; patients should receive sufficient information before testing to make an informed decision to proceed; and both counselling and educational tools should be developed to assist patients in understanding test results, including the personal and family implications.

Isaac Kohane. Genomic tests are, with one notable exception, qualitatively no different from any other clinical test, whether they be electrolyte monitoring, complete blood cell count or cranial magnetic resonance imaging (MRI). Genomic tests, like all other clinical tests, provide a probabilistic measure of certainty that a specific pathophysiological state is present (that is, a diagnosis) or will be present (that is, a prognosis). Whether genomic or conventional, all of these tests are used for clinical decision-making, whether in the context of screening asymptomatic individuals or managing individuals with a complaint. The costs of each of the aforementioned tests, including whole-genome sequencing, are typically less than a couple of thousand dollars, and the volume of data generated is typically not more than a few terabytes. However, the most important difference between genomic tests and clinical tests is that the medical establishment and the medical industrial infrastructure believe them to be very different. This perception is due to a number of well-documented deficiencies in medical education regarding genomic tests[9], discomfort on the part of clinicians in interpreting the genomic tests[10,11] and the complete lack

of infrastructural support in current electronic medical records for the acquisition and processing of genomic and genealogical data[12]. The consequence of these deficiencies has been a remarkable willingness to consider forms of automated decision support and knowledge creation that are, at best, grudgingly accepted for other kinds of testing, mostly because clinicians believe they can use intuition and experience to determine the right time to order conventional clinical tests and are unaware of cognitive biases, such as those caused by insensitivity to the prior probability of diseases[13]. Most clinicians are under no such delusion with regard to genetic testing and, therefore, although the differences are qualitatively minor, the application of principles of rational decision making (for example, maximizing expected utility[14]) are more likely to be applied to genetic tests than to existing clinical tests.

The one area in which genetic tests are qualitatively different is that even a small fraction of an individual's whole genome[15] is highly identifying. This makes the stakes of data security and privacy policies much higher. Genomic data cannot be shared with other researchers without a much higher likelihood of disclosure of identity than with other data types. The risk of disclosure is growing significantly with the accumulation of independent non-medical DNA databases, such as those maintained by the system of criminal justice in the United States.

Sharon E. Plon. Over the past 20 years, the graduating class from my medical genetics fellowship has frequently argued the "what's so special about genetic testing?" question. Technically, genetic tests are handled like other medical tests: they are ordered by a physician on the basis of a medical problem (including family history) and are reported back to a physician for interpretation to the patient. In my experience, however, there are several major differences from other tests. First is the ability of genetic tests to be predictive decades in advance of disease, such that there is a potential likelihood of an adult-onset disorder being identified in a child. Cholesterol levels are predictive of heart disease, but this is categorically different from the near certainty of developing Huntington's chorea if a trinucleotide amplification of greater than 39 repeats is identified[16]. Genome-scale tests also make the likelihood of incidental findings much greater than targeted genetic tests[17] and potentially more than other diagnostic modalities, such as an MRI.

The other unique aspect is the interrelationship of genetic test results among family members. The child with a mutation for an adult-onset disorder identified in a genome-scale test probably inherited it from a parent who is now also at risk for this disease. Importantly, for a single gene or for limited panels of genes, it is often recommended to first test a member of the family with the disease to identify the

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causative mutation in the family before testing at-risk individuals. However, these individuals may have two different physicians and unfortunately may be cared for in two different medical systems. Medical records and insurance companies are currently unable to support family-based genetic analysis. For example, guidelines for *BRCA1* and *BRCA2* genetic evaluation recommend that testing should be initiated with a member of the family with cancer: for example, a woman with end-stage ovarian cancer, not for her own health, but to provide crucial information for her daughters (the guidelines can be found at the US National Cancer Institute website)[18]. However, the physician ordering the test is often required to document medical necessity to obtain insurance coverage of the genetic test for the patient being tested. Similarly, the daughters' insurance companies do not see the reason to fund testing of the mother. A more rational system to handle family-based testing would make genetic analysis much more efficient and cost-effective[19].

Ron Zimmern. Medical test data may be of different types. Not all are, or should be, handled in a similar manner. Clinical genomics data can be considered to derive from what I call 'open-ended tests'. Such tests include the karyotype, X-rays and other imaging modalities and also the clinical examination. They are open-ended because the test may reveal more than the investigator seeks or desires to know. A karyotype may be performed to determine the presence or absence of a trisomy but may show an unexpected translocation. Thoracic imaging may be carried out to look for emphysema but may show an unexpected cancer.

Open-ended tests have always posed a problem to the physician. They show up unexpected or unwanted data that will need interpretation. Clinical judgement as to how best to proceed will be essential. My personal belief is that the problems posed by clinical genomic data are conceptually the same as those posed by any other open-ended test. I am unimpressed with the argument that because they are likely to reveal information to other family members this poses a new ethical problem. All tests will do this to some extent. The diagnosis of cancer in a person doubles the risk in a first-degree relative[20]. If there are ethical issues, they lie in how the patient ought to handle the information rather than whether patient should or should not undertake the test in the first place.

Open-ended tests differ from 'closed' tests, such as uric acid or sodium measurements. Genomic assays may be set up in such a way as to mimic a closed test, such as when specific assays are designed to assay and interrogate variants relating to a particular problem.

Q *On what grounds should findings from genetic tests be returned to patients, and who should decide this? Should we offer patients the choice of whether to sequence certain disease-causing genes?*

L.G.B. These are the core challenges that we face: what subset of the information should be returned and how should we decide what subset this should comprise. It is a given that the patient will need to participate in this decision, but it is also clear that patients do not have the knowledge or tools that they need to make these decisions. I have performed a number of the informed consents for the ClinSeq study[21], and when I tell participants that one of the goals of ClinSeq is to learn what subset of the data they want back, the typical response is "but I want it all". This enthusiasm and optimism is wonderful, but no human being can possibly absorb or use 6 GB of genomic sequence or even 60 MB of exomic sequence. My clinical experience is that a typical patient is information-saturated within 20–40 minutes of discussing a single test result. We have returned unexpected cancer-susceptibility mutations (just one variant per person) to ClinSeq participants, and the only way to summarize the response is to say that they are confused and overwhelmed. These kinds of results can be extracted from the data, they can be returned, and they can be used to alter the management of the patient, but it is emotionally challenging for the patients and needs to be performed thoughtfully.

Clinical genomic data must therefore be stratified and prioritized to bring to the fore the most relevant and useful data for the individual patient and to deliver it to them in a way that allows them to absorb and to use the result. It just is not true that all genetic testing is as laden as is a result for Huntington's disease. Much of it is actually pretty mundane for most patients and could be delivered in creative ways, such as through the Internet or computer-based media, reserving the critical results for delivery in person by a genetics professional. We therefore need to develop an empirical research base on which to determine which results can be returned by which modes for which patients.

W.B. If a genetic finding is generated as a result of clinical testing, practice standards and consensus-based guidelines should determine what results are returned. However, these standards and guidelines do not yet exist, and there is an urgent need to develop them. They should incorporate patient views and respect patient choice, but they must also take into account the appropriate use of clinical resources, including laboratory time and resources involved in generating clinical findings from raw sequence data, and clinician time spent in pre- and post-test counselling[5]. As with other medical resources, patients should not have an unlimited opportunity to request genomic testing results[22]. Most health insurers currently define eligibility criteria for BRCA testing; we are likely to see similar criteria developed in the future to define appropriate clinical use of sequencing tests, including the appropriate range of incidental findings to return.

I.K. The genomic data of a patient should be immediately available to the patient just as all clinical measurements increasingly are. However, under the principles of the Hippocratic corpus "first, do no harm", the interpretation of these data by clinicians should be limited to that subset for which there is a substantive evidentiary base and a quantifiable measure of certainty. In the absence of such a clinical filter on these findings, there will be an overwhelming return of false, misleading and potentially harmful findings, leading to unneeded additional tests and treatments that will make the unnecessary morbidity and costs induced by the prostate-specific antigen screening seem paltry in comparison[23]. In this context, in asymptomatic patients, the prognostic value for most disease is relatively slight, as demonstrated by the recent paper by Roberts *et al.*[24]. Conversely, when there is a clinical indication, the increased prior probability of a pathogenic variant being present substantially decreases the likelihood of false positives and boosts the positive predictive value of testing genes implicated in diseases that might be responsible for the clinical characteristics that the patient is manifesting.

S.E.P. Results from genetic testing have routinely been provided to patients since the advent of clinical cytogenetics in the 1960s, extending to DNA-based linkage in the 1980s and sequence-based tests in the 1990s. Genome-scale molecular tests are the next phase of this realm of medical technology.

Whole-exome tests are now being offered and will clearly improve the diagnosis of patients and families with a wide variety of phenotypes[25–27]. The medical genetics and genetic counselling professions were designed to facilitate appropriate and professional provision of genetic information to patients. Although these professions are small, we should not be in a rush to obviate the use of such professionals for genome-scale testing as there are many challenges ahead, and the possibility of misinterpretation is amplified as the scale of testing increases. By contrast, pharmacogenetic loci may have a more clear-cut medical benefit with less controversy or need for specially trained practitioners for disclosure of the data.

There are several efforts underway to generate a consensus list of medically actionable genes that should always be reported[28]. However, as many of us work to create such lists, they are long (many variants imply actionability if you know enough about them), and conversely many databases currently incorrectly annotate variants as being disease-causative, making fully automated reporting of incidental findings difficult[28]. In my opinion, comprehensive genome-scale reports that include incidental medically actionable variants and/or carrier status should only be provided if the laboratory is provided with clear documentation (namely, consent signed by the patient and the ordering physician or genetic counsellor) showing that the patient understands these possible result types. One will do less potential harm by providing only information that will be adequately interpreted or transmitted. For example, in the case of cancer genomes, the germline genome is being sequenced as a control that is subtracted away before reporting cancer-specific mutations. If laboratories make clear the specific cancer genome test that is being provided, there is no reason to compel them to report actionable germline variants. Performing analysis of the germline data adds costs to the cancer test, including involving laboratory directors with the appropriate germline expertise to avoid inaccurate reports. Research is underway into how often patients (or the parents of patients) want this type of additional germline data interpretation (see the US National Human Genome Research Institute website for details).

R.Z. My default response is to suggest that it is the patient who should decide. But that default position needs to be qualified. The patient seeks an opinion about a specific clinical problem. Technology will allow the investigation of the entire genome (open-ended) to be converted to a conventional (closed) test that looks only for variants that are relevant to that problem. I believe that, at present, with perhaps exceptions for very complex problems (where 'experimental' diagnostic techniques using the entire genome might be used), genomic data should be implemented in the clinical setting as specific arrays directed at specific situations. Interpreting such arrays is time consuming and complex enough. Our logistical, bioinformatic and human resources (and our knowledge) are limited; these barriers speak against the routine use of the whole genome at present.

But, assuming a full genome sequence, the issue of what should be returned to the patient should be a matter for discussion between the physician and the patient. Some will only want information that is relevant to the clinical problem at hand. Others will want information that is related to other unrelated but clinically treatable or preventable conditions, while some will want information about such conditions even if they are not treatable or preventable. The patient's view should be taken into account, but the view of the physician is also important. Clinical judgement must form an important component of the decision-making process. Explicitness and transparency are key, and they must take into account logistics, interpretation and other resource considerations.

The clinical examination will sometimes reveal a problem that is totally unrelated to that for which the patient sought help. It is normal practice, and entirely ethical, for the physician to draw the patient's attention to the new problem and to investigate it appropriately. Variants, even if significant, are in one sense like this but in another sense are not. The unexpected pathogenic variant, unlike the unexpected clinical finding, is indicative not of disease but of the risk of disease. How might that change the approach?

Q *What infrastructure — practical, regulatory, educational and medical — needs to be developed if clinical sequencing is to serve patients' needs best?*

L.G.B. We have essentially none of this in place, and the needs are overwhelming. Building all of these resources simultaneously for the predicted broad uses of sequencing in the clinic is impossible; we cannot justify the cost because we have not yet demonstrated the utility, and we cannot demonstrate the utility until we have the infrastructure. One approach would be to start where it would be easiest to use the technology today, and where it would be most likely to be successful. Currently, this is in the realm of uncharacterized (typically rare) diseases that have eluded the so-called Casablanca diagnostic strategy ("round up the usual suspects"). Such patients are small in number but currently consume large amounts of diagnostic resources in a long (sometimes lifelong) search for a diagnosis. If the disorder is likely to have a Mendelian genetic basis, whole-genome or exome sequencing can end this diagnostic odyssey. If this is where the testing is most likely to be useful today, then we can gradually build a regulatory framework, clinical expertise and training, informatics and legal construct to support that testing and to expand it to more common disorders. We can build the infrastructure to interpret secondary variants, pharmacogenetics, newborn screening, and so on. This 'glass half full' approach exploits the things we know how to do best, instead of focusing on the 'glass half empty' approach, which focuses on the limitations of our data, the fears of ethical calamities and the plain old-fashioned human fear of change.

W.B. We need reliable databases to assist laboratories and clinicians in interpreting the clinical significance of sequencing-based data, and by extension we urgently need the research to populate these databases[5]. Both clinicians and patients will need tools to assist them in interpreting test results. Some of this work can be done on the basis of our current limited understanding of genomics. For example, pharmacogenomic data offer an opportunity to develop point of service guides for clinicians and educational tools for patients[29]. Ultimately, however, the needed infrastructure requires more comprehensive data than we have. Without better information about the clinical implications, clinical sequencing is largely a promissory note.

I.K. The missing infrastructure for genetic tests falls into two categories: the first is technical infrastructure, such as data representation, storage and incorporation into electronic medical records, which will require concerted effort but is conceptually straightforward to implement[30]. The second, less obvious missing infrastructure is the regulatory surround, specifically as pertaining to the identifying nature of genomic data and how this might affect the current flows of patient data for reimbursement, care or research. The biggest problem in

this respect is the broad lack of education in genomic science throughout our clinical establishments, medical schools and training programs[31]. Without this knowledge base and skill set, which includes a well-grounded familiarity with probabilistic reasoning, physicians will not be able to serve as authoritative decision makers for clinical genomics. The recent emergence of a half-dozen whole-genome diagnostic companies suggests that a future infrastructure may in fact be a hybrid of genome-specific interpretive software that is intercalated into the conventional systems used in provider practices. That is, in most practices, the authoritativeness on genomic science will derive from the up-to-date knowledge base and software rather than local expertise. Moreover, it is highly likely that that infrastructure will include a patient-based component so that patients can obtain authoritative advice even when the medical establishment is silent.

S.E.P. With the introduction of any new genomic technology, there is a period where laboratories all set their own standards, and then consensus guidelines are generated. Clearly, guidelines are needed for next-generation test methodologies with regard to the quality of the data needed to call variants and algorithms and the types of data to be reported. The educational need of the medical professionals receiving the reports is proportional to the comprehensiveness of the reporting. If laboratories endeavour to provide reports containing potentially hundreds or thousands of novel or rare variants identified in a clinical exome, this then necessitates the creation of genomicists (physicians who are able to analyse these results) to receive them in order to avoid physicians overcalling rare variants as causative of disease. Experience from *BRCA1* and *BRCA2* testing, where hundreds of thousands of women have been tested, has demonstrated the significant amount of data needed to interpret each new variant appropriately[32]. This emphasizes the need for appropriately curated variant databases and warns against physicians assuming that every rare or novel variant in a disease-associated gene is disease-causing[28]. The educational burden becomes much greater as we try to combine data types or to incorporate low to moderate risk alleles into clinical medicine. We also need to be clear to the public that we are just at the beginning of genome-scale clinical testing; the current tests are not the ultimate test. For example, analysis of the microbiome or sequencing of specific cell types will come online as technology and bioinformatics improve.

R.Z. The organization of clinical services and of laboratory provision, the training of specialists in genomic pathology, genomic medicine and bioinformatics and the education of physicians and other clinical staff will all be needed to prepare for the advent of genomic medicine[33]. Its excitement and potential will need to be balanced against unwarranted hype. The difficulties of interpretation should not be underestimated. The complexities of human biology and the place of epigenetic mechanisms, of the dynamics of gene transcription and translation and of post-translational changes must all be recognized; and this is before considering the influence of the microbiome. The primacy of the genomic sequence is clear, but citizen and physician alike must understand that sequence information will be only one of many determinants of disease and that too great an emphasis should not be placed on it, except when dealing with highly penetrant inherited or heritable disorders. Biology is complex, and interpretation is equally so.

But education aside, the other essential infrastructure will be bioinformatic. Putting all of these data together and making sense of them will require a step change in bioinformatic resources and understanding. Without bioinformatics, there can be no practical way of interpreting the genome for clinical benefit. I would wish to see the establishment of a new speciality, that of the clinical bioinformatician, sitting at the interface between the genomic pathologist and physician on the one side and the academic and mathematical bioinformatician on the other. This will require the establishment of a professional accreditation body and appropriate codes of practice[33,34]. Regulation is of course important, but I am essentially against genetic exceptionalism. All personal medical data should be regulated, but I see no coherent or convincing argument for genomic data to be regulated any differently from any other medical data.

Q *Will sequencing-based diagnostics become routine and fulfil patients' expectations, and what is the most crucial single challenge now?*

L.G.B. The reactions we have struggled with in returning results in ClinSeq are not those of the participants but rather those of some of their physicians. In more than a few cases, the physicians have been dismissive or even angry with us for finding, for example, a mutation that predisposes to cancer in a patient with no personal or family medical history. Our surprise at this reaction emanates from an underappreciated collision of values, priorities and incentives. We feel that a success in ClinSeq is to identify people who have an increased liability towards illness before that illness manifests in them or in their immediate family. Yet, what we are learning is that the busy primary care physician has a hard time fitting this into their practice paradigm, and they are going to need a lot of support, guidance and (to be honest) remuneration to convince them to do this work. This experience has taught us that one of the greatest challenges we geneticists face is to convince our colleagues of the value and utility of a genetic model of preventive medicine but, more importantly, to provide the support and tools to make this approach to medicine a routine part of the health-care landscape.

W.B. Sequencing tests will undoubtedly find a place in clinical care. However, the scope of clinical use is hard to predict at this time. Sequencing-based tests may primarily be used to address specific questions in certain specialities: for example, sequencing of a tumour genome to direct care or use of whole-genome sequencing to evaluate multigenic disorders or to work up an undiagnosed individual with a rare phenotype. Alternatively, sequencing-based tests may achieve a more widespread clinical use. The reason for this uncertainty relates to the most crucial single challenge: a lack of sufficient understanding of the phenotypic implications of sequencing-based data. We need much more research to understand the clinical implications of such data and, by extension, to understand when and how such data can help to improve health care.

Whether sequencing-based tests will fulfil patients' expectations therefore depends on what those expectations are. Genomic information is likely to improve health care in a variety of ways, but it is unlikely to serve as a universal guide to preventing disease, because genetic risk is only one contributor among many to the common conditions that make up most of the disease burden in our society[35]. In the genomics era, we will still need to exercise, to eat prudently and to avoid cigarettes.

I.K. There is little doubt that sequencing-based tests will be routinely used and will indeed be cheaper than many other high-technology tests. Although patients' expectations regarding the oracular capabilities of genomic testing — which have been set by

over a decade of public, grandiose and occasionally portentous forecasts — are unlikely to be met in the context of asymptomatic, healthy patients, they will be exceeded in the individualization of therapy and the recasting of poorly defined disease within a framework of precision medicine[36]. In order to realize this, we have to ensure that, whether it is in the commercial domain or in the public domain, we obtain the most authoritative and up-to-date, continually revised knowledge base as to the clinical meaning of genomic variants. Further, either the medical establishment will rise up to the challenge of the incorporation of this knowledge base into daily practice, or alternative channels for the authoritative application of genomic knowledge will develop and grow.

S.E.P. Genome-scale sequencing tests will rapidly become routine for the diagnosis of children and adults with major medical problems for which there is a very large number of possible Mendelian or high-penetrance genes. The current costs of disease panels — for example, panels of anywhere from 5–50 potentially causative genes — are similar to whole-exome tests. Thus, whole-exome (and subsequently whole-genome) tests are rapidly becoming more efficient. They are likely to become the frontline test for Mendelian or high-penetrance disease-associated mutations. This transition will accelerate as turnaround times shorten and as technology improves for assessing sequence, copy number and rearrangements within the same test. Similar improvements are crucial for cancer exome and genome sequencing to become frontline tests.

There is, however, a very large gap between reporting results for single-gene disorders and understanding how to combine data in a clinically meaningful way for the much larger number of genetic changes that individually have a mild-to-modest impact on disease risk, such as in diabetes. Algorithms for combining gene-by-gene interactions (multiplied many times over) for common variants are far from clinical application and also require the addition of environmental exposures in making disease prediction. An example of this type of work is a very recent study that combined genotyping of 32 common polymorphisms with measurement of sugar-sweetened beverage consumption on obesity outcomes[37]. These types of combinatorial tools are clearly needed for genome-scale testing to be used as routinely as a cholesterol level is today for predicting the risk of heart disease.

R.Z. I hope that certain forms of sequencing tests will become routine. But in this early stage of clinical development, I would suggest that these be confined to specific sequences directed at specific clinical problems, be they developmental delay in childhood, cancers or sets of single-gene disorders. Genome-based diagnosis will have the potential to save resources by preventing the need to undergo phenotype-based tests that more often than not will not lead to a definitive diagnosis. The expectations of the citizen must be reduced for the present but not at the expense of understanding the future potential of this technology. This is a difficult message to market and poses a critical challenge.

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Competing interests statement
L.G.B. declares competing financial interests: see Web version for details.

FURTHER INFORMATION

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