METHODOLOGY

The methodology of the current study entitled "**Precision Nutrition Approach For Prevention and Management of Obesity** " is presented as follows:

3.1 Phase I: Assessment of factors influencing consumer acceptance of genetic testing for personalised nutrition in India

3.1.1 Selection of study participants
3.1.2 Development of validated questionnaire for the baseline survey

3.2. Phase II: Development and Design of algorithm for formulating a gene and gut microbiome based dietary advice

3.2.1 Identification and selection of SNPs associated with obesity.
3.2.2 Development of SNPs based dietary recommendations
3.2.3 Selection of Criteria for assessment of gut microbiome markers
3.2.4 Development of gut microbiome specific dietary recommendations

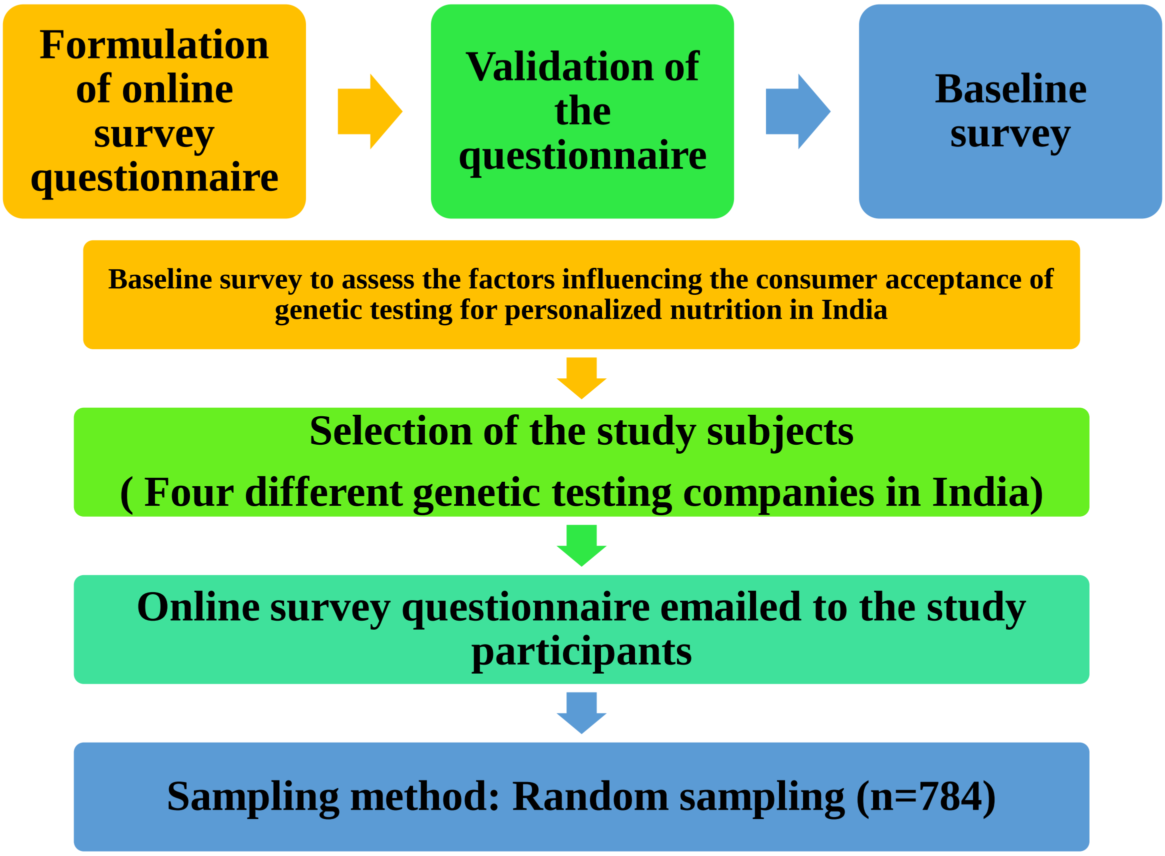
3.3 Phase III: Comparative analysis of precision nutrition vs generic nutrition based dietary advice on long term weight management

3.3.1 Selection of study participants
3.3.2 Assessment of nutritional status of the study participants
3.3.3 Standardisation of protocols for formulating dietary advice for precision nutrition, personalised nutrition and generic nutrition groups.
  3.3.1 Sample collection
    3.3.1.1 Development of the workflow for nutrigenetic testing
    3.3.1.2 Development of the workflow for gut microbiome testing
    3.3.1.3 Interpretation of the nutrigenetic and gut microbiome reports
3.3.4 Evaluation of the efficacy of precision nutrition based dietary advice on long term weight loss
    3.3.4.1 Data collection using diet & lifestyle questionnaire

3.4 Phase IV: Statistical Analysis & Interpretation

The research design of the present study is presented in Figure.17.

Phase I: Assessment of factors influencing consumer acceptance of genetic testing for personalised nutrition in India.



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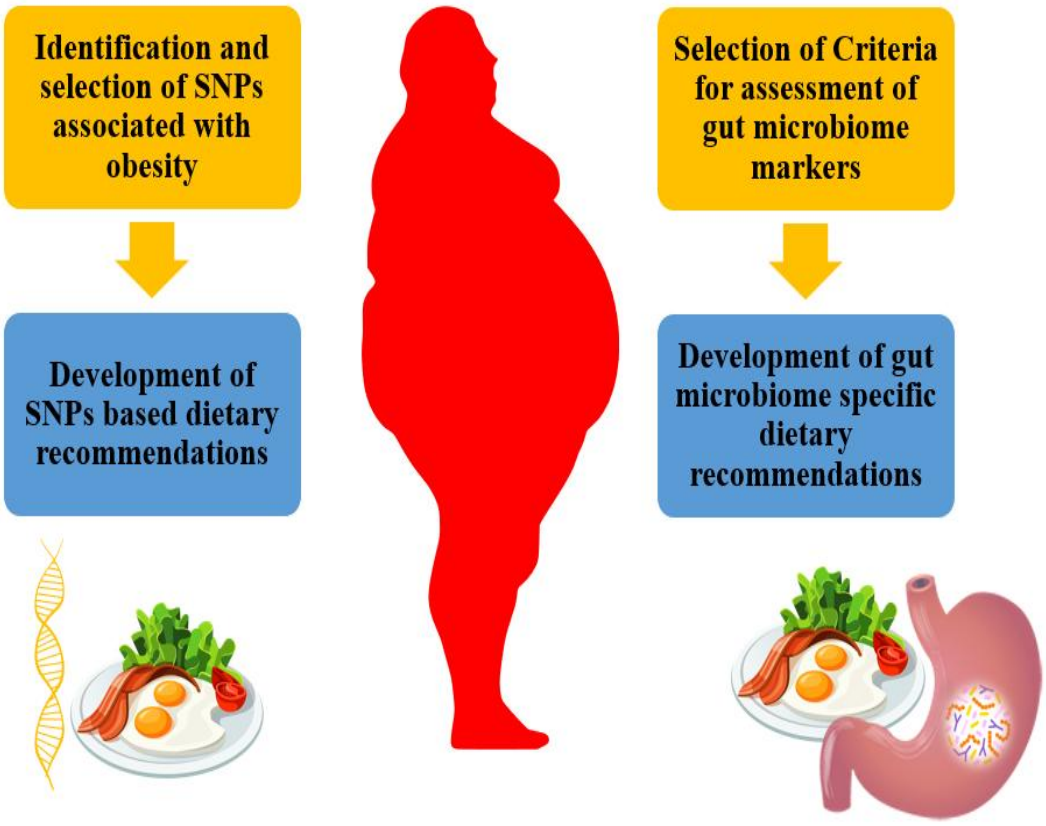
Phase II: Development and Design of algorithm for formulating a gene and gut microbiome based dietary advice for obese individuals



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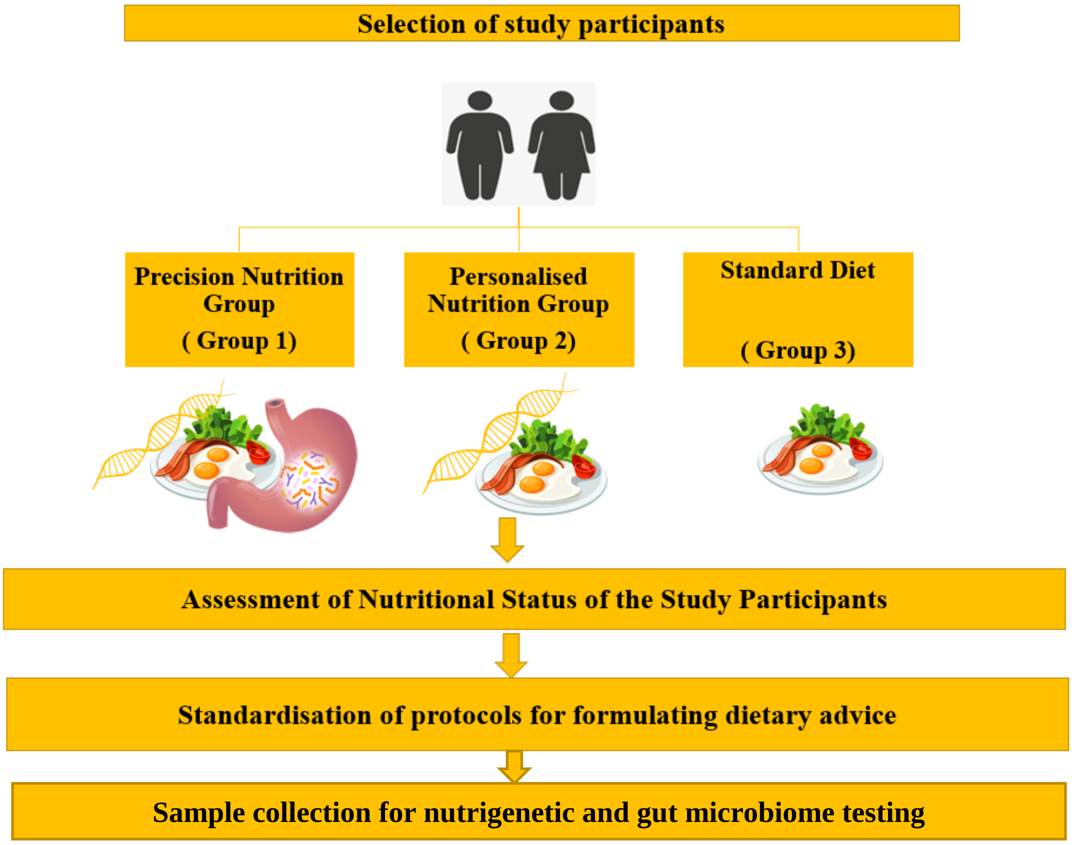
Phase III: Comparative analysis of precision nutrition vs generic nutrition based dietary advice on long term weight management



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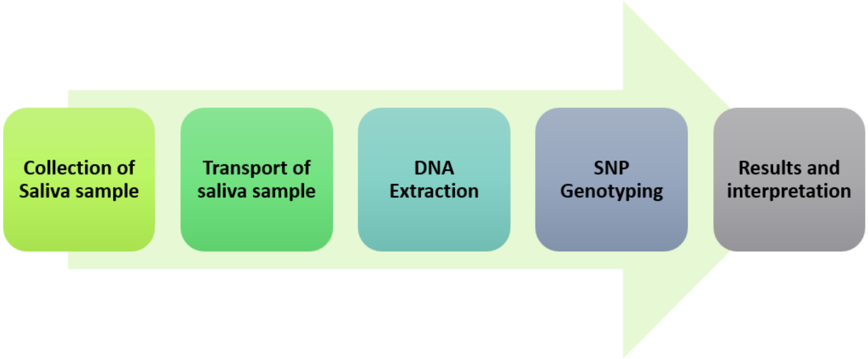
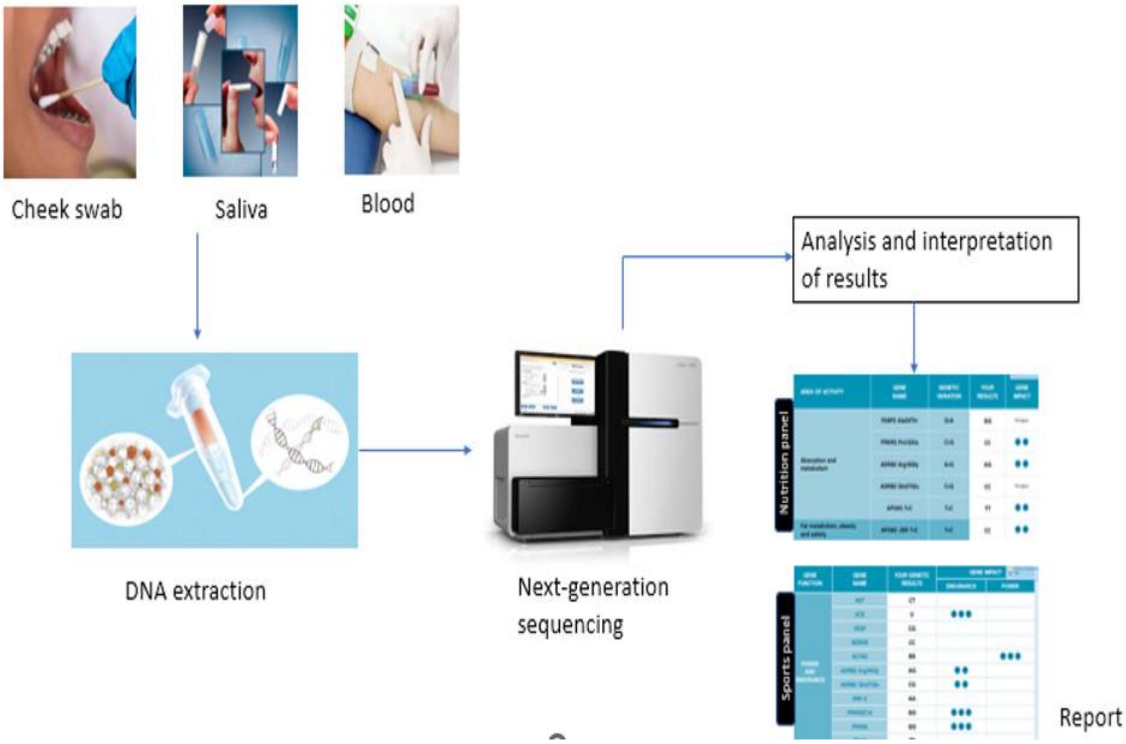
Development of the workflow for nutrigenetic testing
Steps involved in Sample collection



Step by Step Illustration of Workflow of Nutrigenetic Testing



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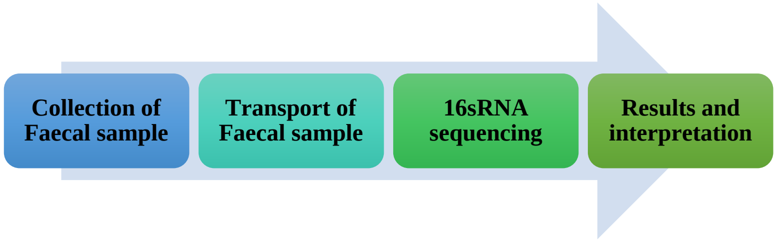
Development of the workflow for gut microbiome testing

Steps involved in Sample Collection



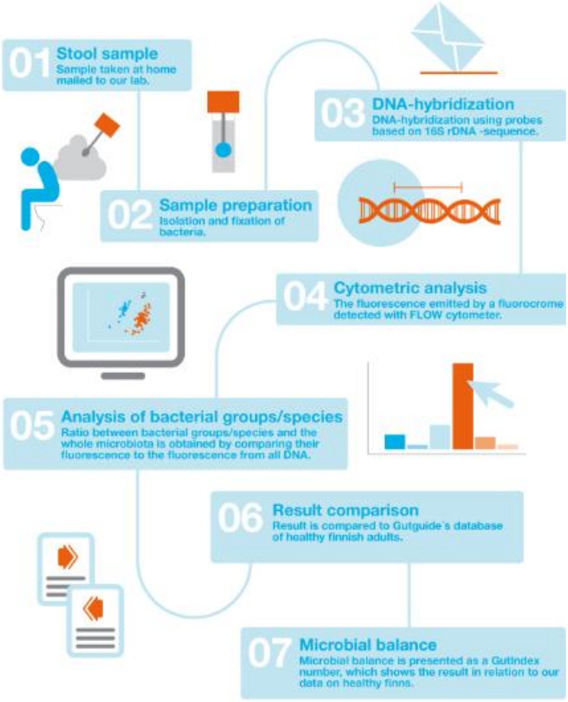
Collection of Faecal sample

Transport of Faecal sample

16sRNA sequencing

Results and interpretation

Step by Step Illustration of Workflow of gut microbiome testing



Evaluation of the efficacy of precision nutrition based dietary advice on long term weight loss

↓

Statistical Analysis & Interpretation

Figure.17 Research Design

3.1.1. Baseline survey to assess the factors affecting consumer acceptance of genetic testing for personalisednutrition

Ethical clearance

Ethical clearance for the research study was obtained from Avinishalingam Institute for Home science and Higher Education for Women, Coimbatore – certificate no. AUW/IHEC/ FSN-19-20/ XPD-36 (Annexure I).

3.1.1.1 Participant recruitment

To assess the factors affecting consumer acceptance of genetic testing for personalised nutrition, a baseline survey was conducted. Random sampling method was used to select the participants for the survey. The following genetic testing companies based out of India were approached to get the consumers contact information who has taken the nutrigenetic test for weight management. Mapmygenome, Swagene, Easy DNA, Xcode Life, The Gene Box, Gene Support, Adam's Genetics. Nutritional Genomix, DNA diet. Out of the nine companies only four consented to send email communications from our behalf, seeking for permission to participate in our study.

Four companies, Easy DNA, DNA diet, Xcode Life and Gene support consented to send out emails to their customers to find about their willingness to participate in the survey. Out of the 783 consumers who consented to participate in the survey, 500 consumers were selected for the study, 125 consumers from each company. Survey Questionnaire was emailed to all the 500 consumers and 483 of them filled the form. Email reminders were sent once in 15 days and whatsapp reminders were sent once in 20 days to complete the online survey questionnaire. The data collection for this survey was carried out between July 2019 till December 2019. A total of 483 consumers completed the baseline survey. Seventeen participants did not complete the survey inspite of regular follow ups.

Flow of participants throughout the survey:

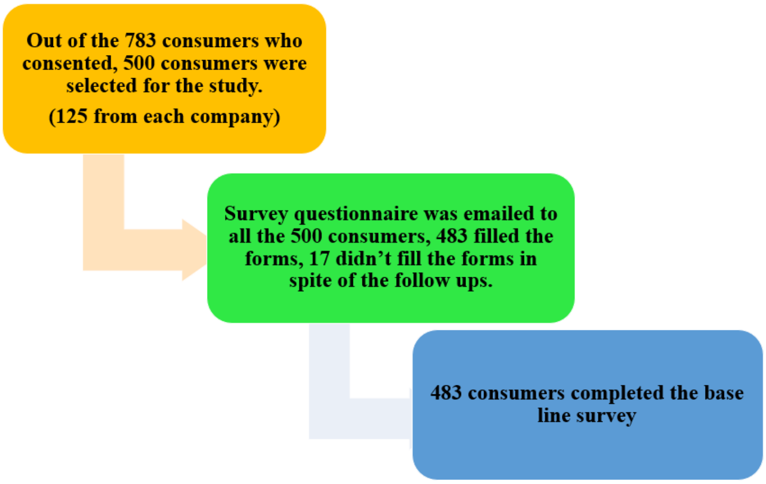


Figure. 18. Flow of Participants throughout the survey

3.1.2. Development of validated questionnaire for the baseline survey

Validated survey questionnaire was adapted and modified from (Szakály*et al.*, 2017) and (Ronteltap*et al.*, 2007), which determined the extent to which an individual's perceptions and attitudes increase the likelihood of acceptance of a genetically based personalized diet. Additional information such as socio-demographic information, other information related to lifestyle data was also included as part of the questionnaire. The survey contained questions related to the potential determinants of consumer acceptance that included attitudes & perceptions, motivation factor, individual's perceptions on sources and genetic information provider, confidence level and utility of DNA based dietary advice.

Ten individuals pretested the developed questionnaire to estimate the time required to complete it, to check the clarity and to evaluate the relevance of the responses. The survey comprised a total of 16 questions; 12 were fixed and 4 were open- ended. Most of the fixed questions were multi-chotomic. The first 14 questions included were aimed to collect personal information and information about the genetic test. The consumer acceptance of genetic testing for personalised nutrition was assessed using 28 statements measured on 5-point Likert- scale, which was modified and adapted from (Ronteltap*et al.*,2007). The questionnaire with 16 questions has been enclosed in Annexure II. Consent information for the participation in the online survey was included in the questionnaire. Descriptive statistics characterized the sample in terms of its demographics, health-related experiences, and other questionnaire responses.The χ2 test was used to test for associations between categorical variables.Statistical significancewas set at $p < 0.05$.

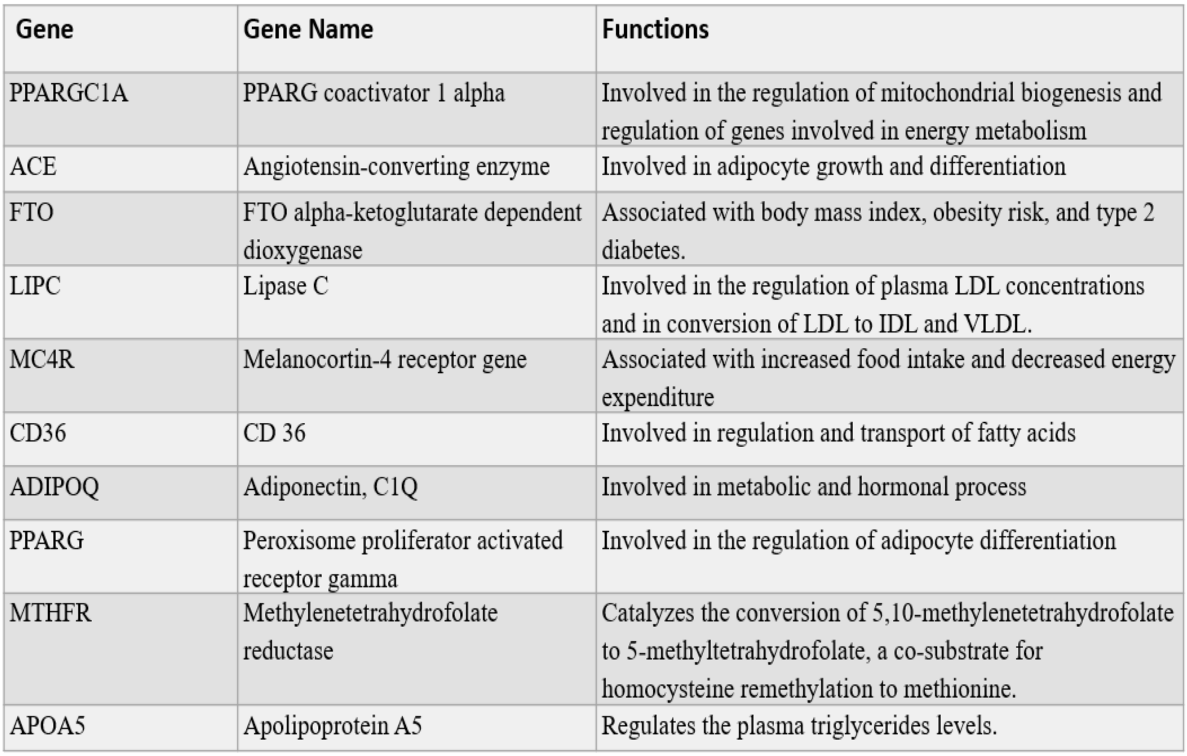
Phase II: Development and Design of algorithm for formulating a gene and gut microbiome based dietary advice for obese individuals.

3.2.1 Identification and selection of SNPs associated with obesity

We selected15 gene variants in 10 genes involved in body weight regulation confirmed by GWAS (Genome Wide Association Studies) to be associated with gene nutrient interactions leading to obesity among Asian Indians. These SNPs are located in or near the FTO, MC4R, CD36, LIPC, ADIPOQ, PPARG, PPARGC1A, MTHFR, ACE and APOA5 genes. All SNPs conformed to Hardy—Weinberg equilibrium. Information about genes and their functions selected for the study are provided in the Table.I

Table I.

Gene and gene functions



Gene	Gene Name	Functions
PPARGC1A	PPARG coactivator 1 alpha	Involved in the regulation of mitochondrial biogenesis and regulation of genes involved in energy metabolism
ACE	Angiotensin-converting enzyme	Involved in adipocyte growth and differentiation
FTO	FTO alpha-ketoglutarate dependent dioxygenase	Associated with body mass index, obesity risk, and type 2 diabetes.
LIPC	Lipase C	Involved in the regulation of plasma LDL concentrations and in conversion of LDL to IDL and VLDL.
MC4R	Melanocortin-4 receptor gene	Associated with increased food intake and decreased energy expenditure
CD36	CD 36	Involved in regulation and transport of fatty acids
ADIPOQ	Adiponectin, C1Q	Involved in metabolic and hormonal process
PPARG	Peroxisome proliferator activated receptor gamma	Involved in the regulation of adipocyte differentiation
MTHFR	Methylenetetrahydrofolate reductase	Catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine.
APOA5	Apolipoprotein A5	Regulates the plasma triglycerides levels.

Source: HUGO (Hugo Gene Nomenclature Committee) (https://www.genenames.org/)

Literature related to these SNPs found in Indian population were systematically reviewed and the mechanistic pathway of these SNPs linked to obesity were studied to develop the rationale for formulating the gene specific dietary recommendations. The list of genes along with their SNPs are provided in Table.II

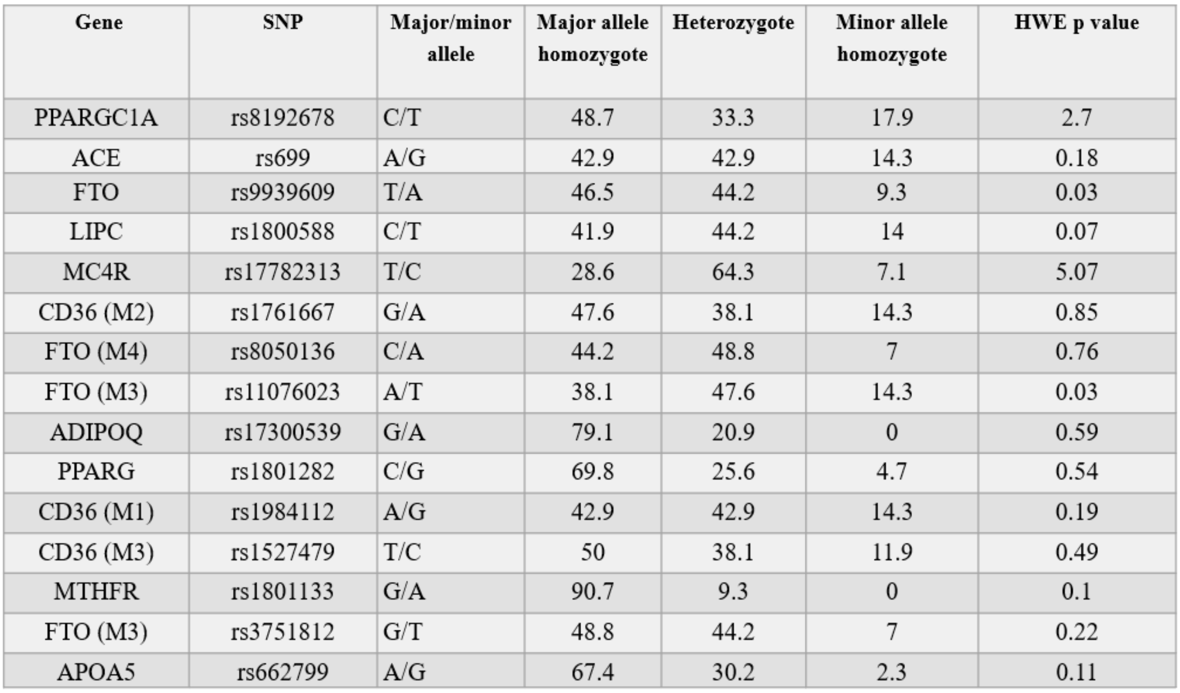
Table II

Rationale for selection of Single Nucleotide Polymorphisms found in Indian population that were associated with obesity

Genes	Gene Name	Rationale for SNP selection	References
PPARGC1A	PPARG Co-activator 1 alpha	Biomarker of insulin secretion and sensitivity in response to glucose challenges.	ZhuLi*et* al., 2017
ACE	Angiotensin-converting enzyme	Codes for the angiotensin-converting enzyme involved in blood pressure regulation and electrolyte balance.	Molina *et* al., 2022.
FTO	FTO alpha-ketoglutarate-dependent dioxygenase	Has associations with obesity, plays a role in controlling feeding behaviour and energy expenditure.	Naaz *et* al., 2019
LIPC	Lipase C	Induces triglyceride-mediated suppression of HDL-C levels.	Verma *et* al., 2016
MC4R	Melanocortin-4 receptor gene	Regulates food intake, energy homeostasis and body weight.	Gao *et* al., 2019.
CD36	CD 36	Facilitator of long-chain fatty acid transport.	Gautam *et* al., 2011.
ADIPOQ	Adiponectin, C1Q	Expressed in adipose tissues, involved in the control of fat metabolism and insulin sensitivity.	Pemmasani*et* al., 2018.
PPARG	Peroxisome Proliferator-Activated Receptor Gamma	Directly regulates the expression of genes involved in lipid transport.	Sanghera *et* al. 2010.
MTHFR	Methylenetetrahydrofolate Reductase	Important for folate and homocysteine metabolism.	More *et* al., 2022.
APOA5	Apolipoprotein A5	Regulates triglyceride and HDL levels.	Chandak *et* al. 2006.

Table III

Frequency distribution of major and minor alleles in the study population



Gene	SNP	Major/minor allele	Major allele homozygote	Heterozygote	Minor allele homozygote	HWE p value
PPARGC1A	rs8192678	C/T	48.7	33.3	17.9	2.7
ACE	rs699	A/G	42.9	42.9	14.3	0.18
FTO	rs9939609	T/A	46.5	44.2	9.3	0.03
LIPC	rs1800588	C/T	41.9	44.2	14	0.07
MC4R	rs17782313	T/C	28.6	64.3	7.1	5.07
CD36 (M2)	rs1761667	G/A	47.6	38.1	14.3	0.85
FTO (M4)	rs8050136	C/A	44.2	48.8	7	0.76
FTO (M3)	rs11076023	A/T	38.1	47.6	14.3	0.03
ADIPOQ	rs17300539	G/A	79.1	20.9	0	0.59
PPARG	rs1801282	C/G	69.8	25.6	4.7	0.54
CD36 (M1)	rs1984112	A/G	42.9	42.9	14.3	0.19
CD36 (M3)	rs1527479	T/C	50	38.1	11.9	0.49
MTHFR	rs1801133	G/A	90.7	9.3	0	0.1
FTO (M3)	rs3751812	G/T	48.8	44.2	7	0.22
APOA5	rs662799	A/G	67.4	30.2	2.3	0.11

The frequency distribution of major and minor alleles in the study population are presented in Table. III. The SNPs were also selected based on our intention to provide dietary advice – those that are involved in energy metabolism, lipid metabolism, associated with eating behaviour, snacking, preference to fatty foods so that it will be comprehensive to formulate the dietary advice for obese individuals. The rationale for developing dietary recommendations for the selected SNPs are furnished in Table. IV

3.2.2 Development of SNPs based dietary recommendations

Genes and their functions were thoroughly reviewed and selected based on its mechanistic pathways linking to obesity. Nutrigenetic or SNPs specific nutrient recommendations were formulated based on the gene function and considering the specificity to the Indian population obesity phenotypes such as Insulin resistance, central adiposity, characteristic thin fat phenotype, lower adiponectin levels, increased triglyceride levels and increased LDL levels. The ten genetic polymorphisms selected for the current study were shown to be present in Indian population and the rationale behind the dietary recommendations are also provided to understand the correlation between polymorphisms and the recommendations used to formulate the SNP specific dietary advice.

Table IV

List of SNPs and evidences associated with nutrient metabolism

S. No.	List of Genetic markers	SNPS	Rationale	Nutrients	Reference
1	PPARGC1A	rs8192678	Polymorphism in PPARGC1A have shown to be associated blood glucose levels	Dietary Carbohydrates	Du *et* al.,2019
,2.	ACE	rs699	Polymorphism in ACE have shown to be associated with hypertension in response to dietary sodium intake	Dietary sodium	Das *et* al., 2008
3.	FTO	rs3751812	Polymorphism in FTO gene have shown to modify obesity related traits such as BMI, Waist circumference in response to dietary fibre	Dietary Fibre	Moore *et* al., 2012
		rs8050136	Polymorphism in FTO gene have shown to modify obesity related traits such as BMI, Waist circumference in response to dietary carbohydrate	Dietary carbohydrate	Karani *et* al.,2016
		rs9939609	Polymorphism in FTO gene have shown to be associated with low satiety and increased eating there by increases the predisposition to weight gain	Calorie dense food	Ningombam*et* al.,2018
4.	LIPC	rs1800588	Polymorphism in the LIPC gene have shown to influence HDL-C concentration in response to dietary carbohydrate and fats	Carbohydrates and MUFA	Ayyappa *et* al.,2013

5.	MC4R	rs17782313	Polymorphism in the MC4R gene is associated with overeating and increased BMI, Waist circumference and reduced insulin resistance	Snacking pattern	Srivastava *et* al.,2016
6.	CD36	rs1761667 rs1984112 rs1527479	Polymorphism in CD3 gene are associated with fatty taste perception	Fatty food	Shanmugamprema*et* al.,2023
7.	ADIPOQ	rs17300539	Polymorphism in the genes coding for ADIPOQ may influence risk of insulin resistence	Response to MUFA	Ramya *et* al.,2013
8.	PRARG	rs1801282	Polymorphism in the genes coding for PRARG gene may also influence carbohydrate intake in response to weight gain	Response to carbohydrate	Phani *et* al.,2016
9	MTHFR	rs1801133	Polymorphism in gene involved in folic acid metabolism have been shown to affect homocysteine levels as well as balance between DNA methylation	Homocysteine levels	Poornima *et* al.,2022
10.	APOA5	rs662799	Polymorphism in apolipoprotein A5 gene are associated with triglyceride levels	Triglycerides levels	Fatma *et* al.,2023

Nutrients associated with these polymorphisms are also presented in the Table IV. SNP specific nutrient recommendations that are developed and used for the current study are provided in the Table. V.

Table.V

SNP Specific Nutrient Recommendations

SNO.	Genes	SNP	Rationale	Nutrient	Recommendation	References
01.	*PPARGC1A*	**rs8192678**	Polymorphism have shown to be associated with increased total , visceral and subcutaneous fat.	Dietary Carbohydrate	Limit your CHO to 50% of your total calories. Resveratrol containing foods such as grapes, red grapes, peanuts, blueberries are suggested to be part of your daily diet, to improve mitochondrial biogenesis.	Du,*et* al. 2019
		rs1801282	Polymorphism have shown to be associated with increased total , visceral and subcutaneous fat.	Dietary Fiber	A diet rich in fruits, vegetables, whole grains, lean protein, and healthy fats, and low in processed and high-calorie foods, may be helpful for weight management and overall health.	Jacob, *et*al.,2016
02.	*ACE*	**rs699**	Polymorphism in ACE have shown to be associated with hypertension in response to dietary sodium intake.	Dietary Sodium	Pomegranates contain natural ACE inhibitor that helps to regulate your blood pressure. Include potassium rich foods such as guava, banana, radish, cherries, pista and pepper. Add spices and herbs to your food to enhances flavor instead of the salt. Limit the amount of canned, processed, baked, salt-dried and pickled foods	Bhagi, *et* al., 2015
03.	*FTO*	**rs3751812**	Polymorphism in the FTO gene have been shown to modify obesity related traits such as BMI , waist circumferences in response to dietary fiber.	Dietary Fiber	High fiber diet will help you to loose weight. Daily inclusion of fiber rich foods is recommended. 30g of dietary fiber is the recommended daily allowance, equivalent to 2 medium sized fruits and 3 serving of vegetables.	Steven, *et* al., 2012
		rs8050136	Polymorphism in the FTO gene have been shown to modify obesity related traits such as BMI , waist circumferences in response to dietary fiber.	Dietary Carbohydrates	Consume a maximum of 50% of daily calories through complex Carbohydrates. Monounsaturated fats help in improving insulin sensitivity. Adding 1 tsp of sesame seeds every day improves your insulin sensitivity. Cinnamon helps to improve your insulin secretion. Add 1 tsp of cinnamon powder to	maleswaran, K. S, *et* al., 2016

					your daily diet. Arginine containing foods such as peanuts, walnuts, chick peas, green gram help to enhance insulin secretion.	
		rs9939609	Polymorphism in the FTO gene have been shown to associated with low satiety & increased eating thereby increase the predisposition to weight gain.	Calories dense foods	Protein and fiber rich snacks can help you to meet satiety (eg. Sundal, egg, veggies, nuts). Try to replace your cab-based snacks to protein or fiber-based snack. Timely spaced meals help to maintain satiety.	Prakash, *et* al., 2016
		rs11076023	Polymorphism have been found to have a heightened preference for high-fat foods, which may contribute to the development of obesity	High fat foods	Diet rich in whole grains, vegetables, fruits, nuts, healthful sources of protein (fish, poultry, beans), and plant oils, while limiting sugared beverages, refined grains, potatoes, red and processed meats, and other highly processed foods, is recommended for weight control	Irgam, *et* al.,2011
04.	*LIPC*	**rs1800588**	Polymorphism in the LIPC gene have shown to influence HDL -C concentrations in responses to dietary CHO and fats	Carbohydrates and MUFA	Inclusion of MUFA rich foods such as sesame , almond , omega 3 , ALA helps to improve insulin sensitivity and HDL-C among these individuals .	Todur, S.P., *et* al., 2013
05.	*MC4R*	**rs17782313**	Polymorphisms have been associated with obesity and related to the regulation of food intake and energy expenditure	Dietary carbohydrate	A diet that is lower in saturated fats and higher in unsaturated fats, as well as a diet that is lower in overall carbohydrate content, particularly refined carbohydrates.	Taylor, e*t* al., 2011
06.	*CD36(M2)*	**rs1761667**	Polymorphisms have been associated with cardiovascular disease and insulin resistance.	Lower Saturated fats and Lower in Total Fat	A diet that is lower in total fat and higher in monounsaturated and polyunsaturated fats, as CD36 is involved in fatty acid uptake and metabolism	Yazdanpanah Zeinab, *et* al., 2017
	CD36(M1)	**rs1984112**	Polymorphism in this gene is involved in fatty acid uptake and metabolism, and	Dietary Fiber	This diet should include a balance of macronutrients, such as whole grains, vegetables, fruits, lean proteins, and healthy fats, while limiting	Farook, *et.* Al., 2012

			it have been associated with cardiovascular disease and insulin resistance.		saturated fats and refined carbohydrates	
	CD36(M3)	**rs1527479**	Polymorphism of CD36 gene variants with metabolic syndrome, type 2 diabetes, oral fat perception, fat preferences, and obesity in various populations.	Dietary Fat	This diet should include a balance of macronutrients, such as whole grains, vegetables, fruits, lean proteins, and healthy fats, while limiting saturated fats and refined carbohydrates	Ashutosh, *et* al., 2014
07	*ADIPOQ*	**rs17300539**	Polymorphism is involved with regulating glucose and lipid metabolism,	Dietary fat	A diet that promotes healthy glucose and lipid metabolism, such as a balanced diet with adequate fiber, lean protein, and healthy fats,	Ramya, *et al.*, 2013
08	*MTHFR*	**rs1801133**	Polymorphism in this gene can affect folate metabolism and increase the risk of DNA damage and oxidative stress	Dietary Folate	A diet rich in leafy green vegetables such as spinach , food rich in B vitamins such as animal product , Avoidances of processed foods such as added sugar and excusive caffeine	Kumar, *et* al., 2016
09	*APOA5*	**rs662799**	Polymorphism in this gene is associated with alterations in blood pressure, serum lipid parameters, and increased risk of metabolic syndrome	Complex CHO	A diet rich in non-refined carbohydrates and low in saturated fats may be beneficial for individuals with the APOA5 rs662799 TT genotype.	Xu, *et* al., 2013

3.2.3 Selection of Criteria for assessment of gut microbiome markers

Direct sequencing of the bacterial 16S rRNA gene has become the most widely adopted method to obtain information with respect to microbiota composition of any given ecosystem, including that of the human gut. This type of microbiota profiling has provided a phylogenetic framework of the gut microbiota. Typical ecosystem features that can be obtained from such analyses include microbial community typing, determining microbial diversity, as well as identifying microbes that are differently abundant between groups of subjects, all of which can be used as targets for microbiome-based classification. Microbial community typing based on 16S rRNA genes includes identifying the presence of community types and the existence of alternative stable compositional states.

Diversity of a microbiota includes the number of different taxa (richness) as well as their (relative) abundance distribution (evenness) within an ecosystem. A high microbial diversity is considered to be beneficial as it is suggested to contribute to resilience after disturbance of the microbiome (Liu,*et al.*, 2020). Indeed, the microbiota diversity is generally higher in healthy subjects than compromised subjects.

The gut microbiome profile of an individual is basically characterised by the following three parameters:

(i) **Composition**
(ii) **Abundance**
(iii) **Diversity**

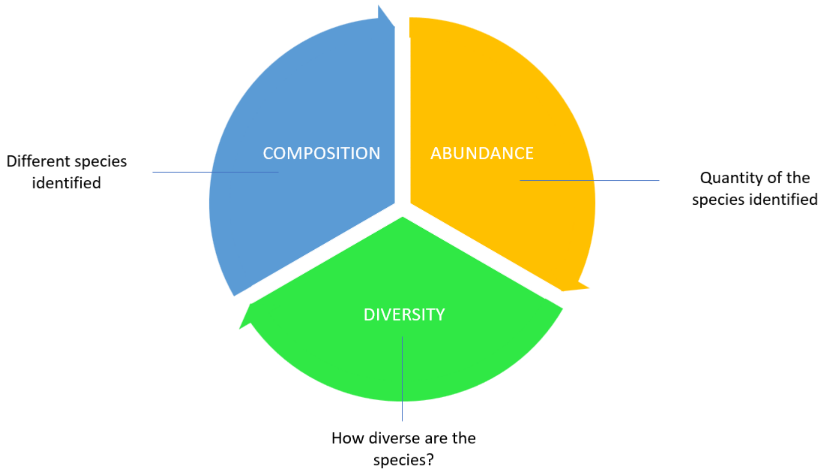


Figure. 19 Determinants of Gut Microbiome Profile

(Liu,*et al.*, 2020).

(i) Composition: The composition of any gut microbiome belongs to 4 major groups of microorganisms called - Bacteria, Archaea, Virus and Eukaryota. For the present study these bacterial sub-groups – actinobacteria, bacteroidetes, firmicutes, proteobacteria were chosen to study the gut composition.

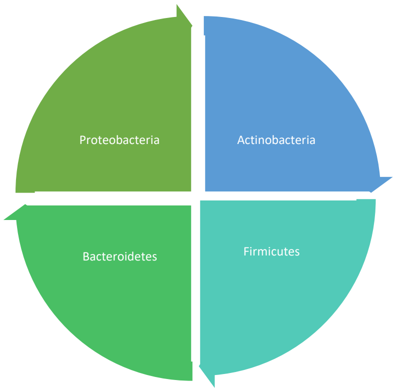


Figure.20 Major Bacterial Sub- groups selected for the study

It is well established that firmicutes were more effective in energy harvest than bacteroidetes, subsequently leading to weight gain. Studies also suggest that altered bacterial composition/ diversity are usually associated with the changes in the gut microbiome composition that influences host health status. The firmicutes/ bacteroidetes ratio has been considered as a possible hallmark for obesity (Bandt, *et al.*, 2007) (Zou, *et al.*, 2020).

The phylum Firmicutes includes bacterial species that are predominantly from the genera Bacillus, Clostridium, Enterococcus, Lactobacillus, and Ruminicoccus, whilst the phylum Bacteroidetes includes bacterial species that are predominantly from the genera Bacteroides, Alistipes, Parabacteroides, and Prevotella(Gibiino, *et al.*, 2018). Probiotics are usually administered to regulate the firmicutes: Bacteroidetes ratio. Table.VII provides the list of probiotics used in the present study that was appropriately chosen based on the gut microbiome profile of the individual.

Table VI

List of Probiotics and their food sources and its influence on the Firmicutes: Bacteroidetes Ratio

	Microbes	**Food sources**	**Firmicutes:Bacteriodetes Ratio**	**References**
1.	Bifidobacterium breve, lactobacillus Johnsonii	Gourd vegetables	Increased firmicutes and decreased Bacteroidetes, Fermented Milk	Sreenivas et al., 2013, Bozzi Cionci*et* al.,2018
2.	Bifidobacterium Infantis	beans, cabbage, brussels sprouts, broccoli, asparagus	Increased firmicutes and Bacteroidetes	Yazawa & Tamura, 2018
3.	Lactobacillus reuteri	Kefir, yogurt	Increased firmicutes and Bacteroidetes	Slattery *et* al., 2019 Hekmat *et* al.,2009
4.	Lactobacillus Casei	Fermented milk, blackberry	Increased Firmicutes and Bacteroidetes	Millette *et* al.,2007
5.	Lactobacillus rhamnosus	blackberry	Increased Bacteroidetes and decreased Firmicutes	Yang *et* al.,2014
6.	Lactobacillus brevis, Lactobacillus gasseri	Fermented vegetables, olives and pickles, kimchi	Increased Bacteroidetes and decreased Firmicutes	Teixeira (1999)
7.	Leuconostocmesenteroides		Increased Bacteroidetes and Firmicutes	Yang *et* al., 2014

(ii) Ways to increase the abundance and diversity of the gut microbiome:

According to several studies, these bacterial genus and species have been associated with beneficial health marker, performs important functional roles and have been negatively associated with various diseases including obesity. The following bacterial species were considered for the study, Akkermansiamuciniphilasp, bifidobacterium sp., Eubacterium sp., Roseburia sp. and Faecalibacteriumprausnitzii sp.

3.2.4 Development of gut microbiome specific dietary recommendations

The gut microbiome specific dietary recommendations were formulated taking into consideration to maintain gut microbiome composition, increase diversity and abundance of species. According to several studies, these bacterial genus and species havebeen associated with beneficial health marker,obtain importantfunctional roles and have been negatively associated with various diseases including obesity. The following bacterial species were considered for the study, Akkermansiamuciniphilasp, Bifidobacterium sp., Eubacterium sp., Roseburia sp. and Faecalibacteriumprausnitzii sp. The rationale for choosing these species are research evidences that have established the role of these species with respect to health and in obesity.

(i) **Akkermansiamuciniphila sp.,** is found to have a symbiotic relationship with the host. When Akkermansia breaks down mucins, it turns them into short-chain fatty acids (SCFAs), including acetate, acetate is used by other beneficial bacteria such as Faecalibacteriumprausnitzii, to make butyrate, which is an essential energy source for the cells present in the gut via the process called cross- feeding. This group of species plays a key role in butyrate production.

(ii) **Faecalibacteriumprausnitzii sp**. is one of the main butyrate producers. These molecules are an important energy source for the body, providing between 5 to 15% of a person’s daily caloric needs.

(iii) **Roseburiasp.**functions as an important butyrate producer to generate energy. In the body, Roseburia secretes various molecules that then interact with the host and other bacteria in the gut. These included short chain fatty acids (SCFAs) such as acetate, propionate and butyrate, which are produced after the fermentation, or breaking down, of indigestible dietary carbohydrates.

(iv) **Bifidobacterium sp.**Bifidobacteria typically make up less than 10% of the bacteria in the adult gut microbiome. One of the main functions of this type of bacteria in humans is to digest fiber and other complex carbohydrates. Fiber has been shown to help reduce weight gain and the risk of diabetes, heart disease and other chronic disorders. Bifidobacteria may help reduce the risk of these diseases by digesting fiber. It contributed to the production of SCFA’s and other important chemicals including B vitamins and healthy fatty acids.

(v) **Eubacteria sp.** These are involved in SCFA production to form beneficial phenolic acids. (Lordan et al.,2019) Table.VII presents the gut microbe specific dietary recommendations.

Table VII

List of Gut Microbes and their Functions and recommendations used for the study

Species	Functional Role	Dietary Recommendations	References
Akkermansiamuciniphilia sp.	Modulates basal metabolism, reduces endotoxemia and prevents atherosclerosis, enhances SCFA production, prevents hyperlipidemia by clearing lipid intermediates, restores epithelial tight junction proteins, and prevents liver disorders.	Include prebiotics (bananas, whole grains), and fructooligosaccharides; following a FODMAP diet may increase the abundance of *A. muciniphilia* (foods to limit: wheat, rye, cashew, pistachios, onion, garlic, cabbage, prunes, apple, pear, watermelon, coconut water); polyphenols like grapes may also increase their abundance.	Xu *et* al., 2020 and Zhou *et* al., 2017.
Faecalibacteriumprausnitzii sp.	Anti-inflammatory potential in IBD, restores gut barrier in diabetic conditions, enhances butyrate production, reduces microbial translocation.	High-fibre diet with less meat, inclusion of prebiotics, inulin-type fructans (banana, garlic, onions, wheat bran), and fructo-oligosaccharides.	Xu *et* al., 2020 and He *et* al., 2021.

Roseburia sp.	Prevents intestinal inflammation and maintains energy homeostasis by producing metabolites, regulates barrier homeostasis, and cytokine release through its metabolite butyrate. Its role has been implicated against disorders of the nervous, digestive, respiratory, circulatory system, and metabolic, autoimmune diseases.	Food sources to include: coconut palm, tomato, legumes.	Nie *et* al., 2021 and La Rosa *et* al., 2019.
Eubacterium sp.	Enhances butyrate formation, maintains intestinal metabolic balance, modulates gut inflammation, facilitates metabolic improvement in insulin-resistant individuals, and mediates anti-cancer effects through butyrate.	Include sources of polyphenols, cocoa flavanols, and cranberry.	Engels *et* al., 2016 and Lordan *et* al., 2019.
Bifidobacterium sp.	Has anti-mutagenic activities against colorectal cancer, acts against diarrhoea, reduces symptoms of IBD, produces health-promoting metabolites – conjugated linoleic acid and bacteriocins.	Include fermented dairy products (curd, buttermilk, cheese), prebiotics, and non-digestible oligosaccharides (galactooligosaccharides – dairy products, beans, root vegetables; fructooligosaccharides).	O’Callaghan *et* al., 2016 and Arboleya*et* al., 2016.

3.3 Phase III: Comparative analysis of precision nutrition vs generic nutrition based dietary advice on long term weight management

3.3.1 Selection of study participants

For the present study, healthy men and women with BMI ≥ 25- 30 kg/m^2 visited Geneobe Weight Loss Clinic, Coimbatore, India was selected. The participants were divided into three groups namely, group-1, precision nutrition group (N=50), Where the participants received SNP based and gut microbiome specific dietary advice, group -2, personalised nutrition group (N= 54), the participants received only SNP based dietary advice and the group -3, standard diet group (N=52) where the participants received generic dietary advice. All the participants who had enrolled for the study were given a baseline diet of 1500 kcal.

3.3.2 Assessment of nutritional status of the study participants

Nutritional status of all the study participants were assessed using a lifestyle and health risk assessment questionnaire. The health and lifestyle assessment questionnaire is attached in Annexure III. Information related to personal information, anthropometric measures such as BMI, WC, body measurements were collected. Questions related to the previous weight loss history, eating pattern, reasons for weight gain, medical history, current medical conditions, medication was also included as part of the questionnaire. A brief diet recall was also obtained from each participant of the study to know their dietary preferences, meal timings and emotional eating cues. Food frequency consumption was also noted as a part of this questionnaire. Clinical signs and symptoms were also assessed and included as a part of this questionnaire.

A snapshot of a sample diet recall collected from the study participant is presented in Plate I.

Time	Meals	Quantity	Comments
6:30 am	Wake up		
7:00 am	1 cup of coffee Idly Coconut chutney	150ml ½ 2 tbsps	Frequent burping noticed.
1:00 am	Rice Rasam Sambar Dal Brinjal poriyal	1.5 cups 75ml 50ml 2 tbsps 40g	Feels full after the meal
7:30 pm	Upma Onion chutney	75g 2 tbsps	
9:00 pm	1 glass of milk	150ml	

Plate 1. Snapshot of sample diet recall collected from the participants in the study

The questionnaire was developed to assess the ABCD's - anthropometry, biochemical, clinical signs and symptoms and diet recall to determine the nutritional status of the participants of the study.

3.3.3 Standardisation of protocols for formulating dietary advice for the study participants

The protocols for developing the dietary advice for each study group was standardised based on the following guidelines presented in Table.VIII. Diet and lifestyle information about the participants was collected using the questionnaire. Based on the diet recall, genotype-based information and gut microbiome results dietary recommendations are customised.

Table VIII

Standardisation of Protocols for Formulating Dietary Advice for the Study Participants

Group	Dietary Guidelines	Follow up
Group -1 Precision Nutrition Group	1500 Kcal Baseline Diet + SNP based recommendations (10 genetic markers used) (FTO, MC4R, CD36, LIPC, ADIPOQ, PPARG, PPARGC1A, MTHFR, ACE and APOA5 genes) + Gut microbiome specific recommendations	30 days, 60 days and 90 days.
Group -2 Personalised Nutrition Group	1500 Kcal Baseline Diet + SNP based recommendations (10 genetic markers used) (FTO, MC4R, CD36, LIPC, ADIPOQ, PPARG, PPARGC1A, MTHFR, ACE and APOA5 genes)	30 days, 60 days and 90 days.
Group-3 Standard Diet Group	1500 Kcal Baseline Diet	30 days, 60 days and 90 days.

A sample 1500 kcal baseline diet is attached (Plate II). The standard dietary guidelines using RDA values were used for calculations for formulating the diet for the Standard Diet - group 3 (Figure.5). For participants from group 1 and group 2, the base line diet was customised further using the SNPs and gut microbiome specific dietary recommendations.

Plate II. Sample of Baseline Diet – 1500 Kcal

Day 1	**Day2**	**Day 3**	**Day4**	**Day 5**	**Day 6**	**Day7**
1 glass of warm water with fenugreek seeds or powder	1 glass of warm water with 1 tsp of cinnamon powder	1 glass ofwarm water with fenugreek seed powder	1 glass of warm water with 1 tsp of cinnamon powder	1 glass ofwarm water with fenugreek seeds	1 glass of warm water with 1 tsp of cinnamon powder	1 glass of water with fenugreek seeds
Cinnamon powder and fenugreek sees helps to improve insulin sensitivity and maintain blood sugar levels.						
Before workout: Have nuts (10 almonds + 10 cashews) + ½ apple blend it into a shake (40 minutes before exercise) or musk melon or water melon.						
Pista-4+ 1 fig	Walnuts-4+ 1 dates	Almonds-4+ 1 figs	Walnuts-4+ 1 dates	pista-4 + 1 dates	Walnuts-4 + 4 raisins	Almonds-4+ 4 raisins
After work out: 1 glass of bottle with few drops of lemon (4-5 drops) + 4-5 mint leaves)						
2 idly with sambar- 1 cup and chutney+ 1 handful of boiled chick peas.	2 chapathi + 1 cup of dal + 1 cup of vegetable	2 millet dosa + 1 cup of sambar+ 1 cup of vegetable	1 bowl of ragi porridge (in thick form) + 1 glass (50ml of almond milk)	3 multi dal vadai with sambar – 1 cup	½ cup millet Pongal (1 handful)+ 1 cup of veg gravy/ sambar+ 1 fruit	1 bowl of chick peas (steamed and made into sundal) + 1 dosa+ 1 tbsp of chutney
It is suggested that your plate has more proteins than carbs and less fat(Moderate carb, adequate protein diet is suitable to your body type).						
1 glass of water with 1 tsp of chia seeds						
1 amla	1 apple	1 cucumber	1 cucumber	1 carrot	10 almonds	
Lunch: should include either 1 cup of yoghurt or 1 glass of butter milk.						
1 boiled carrot (either as such or as a soup) **1 cup of rice (keep alternating the rice that is used, millets, broken wheat, brown rice)** **1 cup of spinach** **1 cup of rasam (garlic and pepper to be added every day)** **1 cup of lentils/ pulses (rajmah, chick peas, karamani, soya beans)** **1 glass of buttermilk or curd**						
1 cup of spinach to be added to your daily diet. (either in the form of soup or poriyal). Carrots to be added every day. 4 garlic roasted to be included along with your meals.						
Snack : as you prefer (masala puffed rice / peanuts or cashews roasted/ pine nuts – 3 tsps/ pumpkin seeds – 1 tbsp)						
Millet Pongal -1 cup with sambar	Rava dosai -1 with stuffed veg+ chutney	Kolukattai – 4 (small with peanut chutney	Idiyappam – 2 slices with coconut milk or veg kurma	Wheat upma with lot of veg with coconut chutney – 1 handful+ 1 tsp	1 cup of curd rice with veg with 1 cup of veg	1 cup of ravapongal with sambar-1 cup+ 1 cup of veggetables
1 small fruit to be served along with the dinner						
¼ cup of milk with black pepper	¼ cup of milk with ½ tsp of turmeric	¼ cup of milk with black pepper	¼ cup of milk with ½ tsp of turmeric	¼ cup of milk with black pepper	¼ cup of milk with ½ tsp of turmeric	¼ cup of milk with black pepper

3.3.1 Sample collection

3.3.1.1 Development of the workflow for nutrigenetic testing

For nutrigenetic testing, Geneobe sample collection kits was used. Saliva sample was collected at the clinic and participants completed a comprehensive diet and lifestyle questionnaire. The saliva samples were sent by courier to Xcode Life sciences and genetic testing was carried out using aExome sequencing method. Variants of 10 genes with 15 gene variants were tested. The workflow as illustrated in the research design (Figure.1) was developed for nutrigenetic testing of the participants who enrolled in the study.

3.3.1.2 Development of the workflow for gut microbiome testing

For gut microbiome testing, faecal sample collection kits were used. Faecal sample was collected at the participants home. The faecalsamples were sent by courier to Bug speaks company in Bangalore, India and the gut microbiome testing was done using 16sRNA sequencing. The workflow as illustrated in (figure.1) which was developed for gut microbiome testing of the participants who enrolled in the study.

3.3.4 Evaluation of the efficacy of precision nutrition based dietary advice on long term weight loss

3.3.4.1 Data compilation using diet & lifestyle questionnaire and the results from the nutrigenetic and gut microbiome tests.

Information related to the diet and lifestyle of the participants were collected by using the questionnaire and recorded. The nutrigenetic test analyzed the 10 genetic variants and the gut microbiome test revealed information about the gut microbiome related markers associated with obesity. This data was then compiled and used to formulate the dietary advice for the different groups in the study.

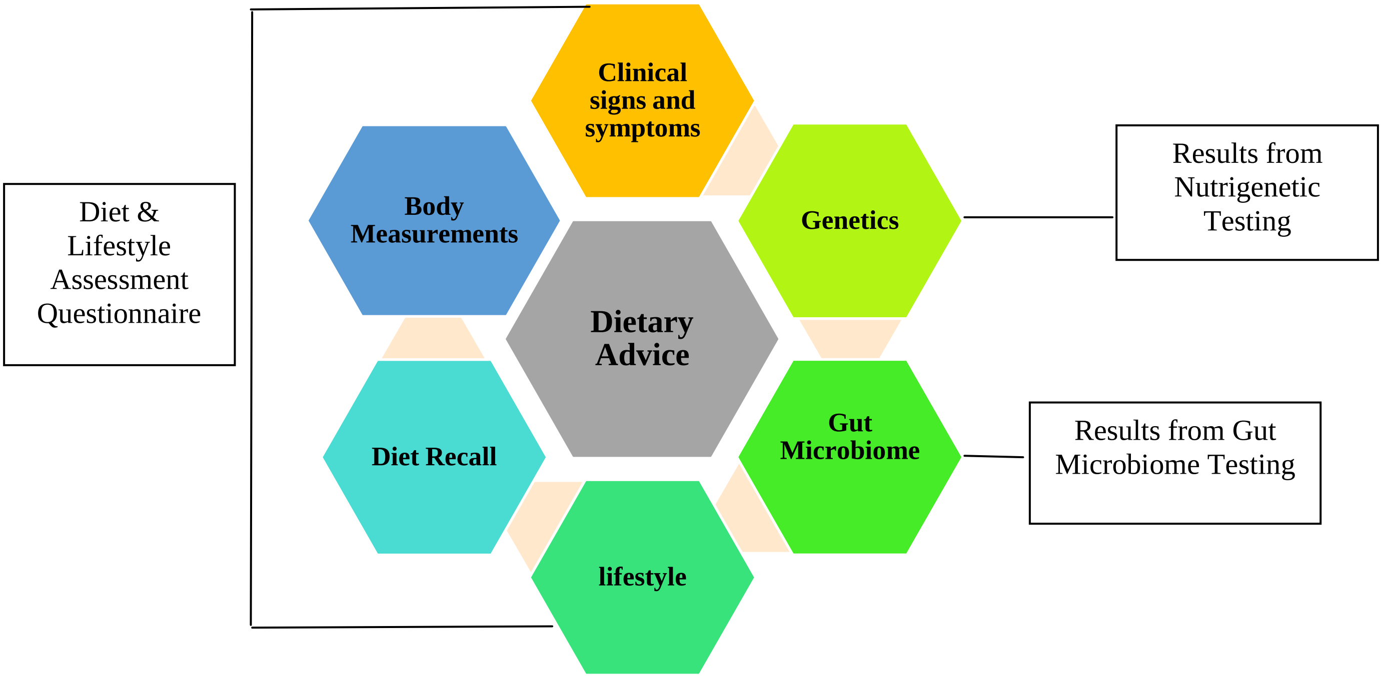


Figure.21 Pictorial Representation of Different Components of Dietary Advice Used for the Study

The case histories of 50 participants in the precision nutrition group (22 females, 28 males) were compared to those of 52 participants in the personalised nutrition group (23 females and 29 males) and 54 participants in the standard diet group (20 females and 34 males). Participants were followed-up at 30 days, 60 days and 90 days respectively. Body weight, BMI and waist circumference were measured and recorded during their clinic visits. The data collected from the three groups were analysed to study the impact evaluation.

3.4 Phase IV: Statistical Analysis & Interpretation

The findings of the preliminary survey to assess the consumer acceptance of genetic testing for personalised nutrition were statistically analysed to assess percentage, standard deviation and association (p-value analysis) of the data collected. Percentage analysis was done to statistically assess various attributes of the survey and multinomial logistic regression analysis was conducted to determine the degree to which the current medical condition of the participants predicted willingness to undergo genetic testing and/or follow a personalised diet. Baseline characteristics of the participants of the survey and their attitudes, perceptions to consumer genetic testing for personalised nutrition was analysed using standard deviation, Significance test using p-value and percentage analyses. Categorical variables were showcased as number and percentage.

Normally distributed continuous variables were presented as mean with SD. Repeated measures ANOVA was selected to analyse the changes in the diet composition and dietary intakes between three study groups. Statistical analyses were conducted using SPSS Version 20.0 for Windows (IBM Corporation ARMONK, NY, USA).

The results obtained from the efficacy analysis of the dietary advice were analysedmainly using percentage analysis, standard deviation, paired samples test (t-value) and level of significance (p-value). Univariate analysis with cumulative percentage was applied to find out the efficacy of the dietary advice given to study participants. Odds ratio was calculated for the change in weight loss among three study groups.

Apart from cumulative percent analysis, exploratory statistical method was used in the study as it utilised variable scoring. The gathered data contained both aspects related to qualitative and quantitative parameters. Hence, the study was undertaken with both these techniques for the data analysis. Advanced exploratory tool for the statistical analyses were compartmented into two sections. The first stage assessed the reliability and validity of the measure applied in this study and examined the descriptive statistics of the measurement items. That is, the initial stage scrutinized the descriptive data of the measurement objects and appraised the dependability (reliability) and strength (validity) of the measures administered in the current study. The subsequent phase evaluated the projected representation of research and this consisted of gauging the contributions and relevance of the evident variables path coefficients. In other words, the second stage involved assessing the contributions and significance of the manifest variables path coefficients tested the proposed research model and this enabled testing of the proposed research model. The data analysis was done with SPSS 20.0 for Windows OS. The attributes of the data gathered from the participants were described and summarized using descriptive statistics.

Parametric statistics like one way ANOVA and Z-test were used for comparison of the factors considered between different levels of the demographic variables. Testing the normality of the data before conducting any statistical analysis forms very much crucial as the statistical procedures and tests differ for normal data and non-normal data.